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(54) Title: TRANSDUCTION OF RECOMBINASES FOR INDUCIBLE GENE TARGETING

(57) Abstract: The present invention provides the use of a fusion protein comprising a site-specific DNA recombinase domain and a protein transduction domain for preparing an agent for inducing target gene alteration in a living organism or in cultured cells, suitable fusion proteins and a method for theproduction of said fusion proteins.

Transduction of recombinases for inducible gene targeting

The present invention provides the use of a fusion protein comprising a site-specific DNA recombinase domain and a protein transduction domain for preparing an agent for inducing target gene alteration in a living organism or in cultured cells, suitable fusion proteins and a method for the production of said fusion proteins.

Background

For some years targeted mutagenesis in totipotent mouse embryonic stem (ES) cells has been used to inactivate genes, for which cloned sequences were available (Capecchi, Trends in Genetics 5, 70 - 76 (1989)). Since ES cells can pass mutations induced in vitro to transgenic offspring *in vivo*, it is possible to analyze the consequences of gene disruption in the context of the entire organism. Thus, numerous mouse strains with functionally inactivated genes ("knock out mice") have been created by this technology and utilized to study the biological function of a variety of genes.

A refined method of targeted mutagenesis, referred to as conditional mutagenesis, employs a site-specific recombination system (e.g. Cre/loxP or Flp/frt – Sauer and Henderson, N. Proc. Natl. Acad. Sci. USA *85*, 5166-5170 (1988); Senecoff et al., J. Mol. Biol., 201, 405 - 421 (1988)) which enables a temporally and/or spatially restricted alteration of target genes (Rajewsky et al., J. Clin. Invest., 98, 600 - 603 (1996)). The creation of conditional mouse mutants requires the generation of two mouse strains, i.e. the recombinase recognition strain and the recombinase expressing strain. The recombinase recognition strain is generated by homologous recombination in ES cells as described above except that the targeted

exon(s) is (are) flanked by two recombinase recognition sequences (hereinafter "RRS"; e.g. loxP or frt). The type of recombination event mediated by the recombinase depends on the disposition of the RRS, with deletions, inversions, translocations and integrations being possible (Torres and Kühn, Oxford University Press, Oxford, New York (1997)). By placing the RRS into introns, an interference with gene expression before recombination can be avoided. The recombinase expressing strain contains a recombinase transgene (e.g. Cre, Flp) whose expression is either restricted to certain cells and tissues or is inducible by external agents. Crossing of the recombinase recognition strain with the recombinase expressing strain recombines the RRS-flanked exons from the doubly transgenic offspring in a prespecified temporally and/or spatially restricted manner. Thus, the method allows the temporal analysis of gene function in particular cells and tissues of otherwise widely expressed genes. Moreover, it enables the analysis of gene function in the adult organism by circumventing embryonic lethality which is frequently the consequence of gene mutation. For pharmaceutical research, aiming to validate the utility of genes and their products as targets for drug development, inducible mutations provide an excellent genetic tool. However, the current systems for inducible recombinase expression in transgenic animals suffer from a certain degree of leakiness in the absence of the inducer (Kühn et al., Science 269(5229):1427-9 (1995); Schwenk et al., Nucleic Acids Res.; 26(6):1427-32 (1998)). Furthermore, the generation of conditional mutants is a time consuming and labor intensive procedure, since the recombinase recognition strain and the recombinase expressing strain have to be breed at least over two generations in order to obtain animals carrying both, the recombinase transgene and two copies of the RRS-flanked target gene sequence.

Protein tranduction domains (hereinafter shortly referred to as "PTD") that have the ability to cross cell membranes were identified, e.g. in the

Antennapedia protein from *Drosophila* (Vives et al., J. Biol. Chem, 272(25):16010-7 (1997)), Kaposi fibroblast growth factor (Kaposi FGF; Lin et al., J. Biol. Chem. 270: 14255-58 (1995)), VP22 from HSV (Elliott and O'Hare, Cell, 88(2):223-33 (1997)) and TAT from HIV (Green and Loewenstein, Cell, 55(6):1179-88 (1988); Frankel and Pabo, Cell, 55(6):1189-93 (1988)). WO 99/29721 moreover mentions TAT mutants having an enhanced activity as compared to the wild-type peptide.
Fusion of PTDs to heterologuous proteins conferred the ability to transduce into cultured cells (Fawell et al., Proc. Natl. Acad. Sci. USA, 91(2):664-8 (1994); Elliott and O'Hare (1997), Phelan et al., Nature Biotech. 16; 440-443 (1998) and Dilber et al., Gene Ther., 6(1):12-21 (1999)). Dalby and Bennett showed that a fusion protein consisting of VP22 and functional Flp recombinase translocated between cells in culture (from COS-1 cells transfected with VP22-Flp to CHO cells carrying Flp recognition sites (FRT sites); see Dalby and Bennett, Invitrogen, Expressions 6.2, page 13 (1999)). Further WO 99/11809 mentions a fusion protein Antp-Cre and emphasizes that it may be used to deliver the Cre into the cell which recombines inside the cell nucleus. It is mentioned that the fusion protein is suitable for manipulating genomic DNA at precise locations in a temporal regulated manner.

Furthermore, a recent report demonstrated that the ß-galactosidase protein fused to the 11 amino acids PTD from the HIV TAT protein can infiltrate all tissues of living mice reaching every single cell (Schwarze et al., Science, 285(5433):1569-72 (1999)). Finally, WO 99/60142 discloses vector constructs for gene therapy carrying a tumor cell sensitizing gene, a sensitizing gene expression regulatory system, a control gene and a control gene expression regulatory system, wherein the control gene can be a fusion gene consisting of a recombinase (viz. Cre or Flp) and a trafficking protein (viz. VP22).

With regard to the fusion protein Antp-Cre of WO 99/11809, it is however, general knowledge in the art that the Antennapedia PTD is not a generally applicable transducing protein, namely it has only a limited activity with proteins having more than 100 amino acid residues (Derossi et al., Trends Cell Biol. 8: 84-87, 1998). In view of the limited transducing activity of the Antp PTD and the size of the generally known recombinases (ranging from about 200 to about 600 amino acid residues), it was desirable to provide a more potent system for the transduction of recombinases. It was, however, not clear for a person skilled in the art whether PTDs would be effective at all with recombinases for the following reasons:
(i) only a single example of PTD-mediated delivery of proteins (above 100 amino acid residues) *in vivo* has been reported so far (Schwarze et al., Science, 285(5433):1569-72 (1999); Fawell et al., PNAS, 91: 664-68 (1994); both references describing the TAT-mediated transduction of ß-galactosidase in mice);
(ii) it is known that - due to defolding and refolding processes - the transduction of native proteins into cells may result in a significant loss of protein activity (e.g., as described for TAT-GFP; Schwarze et al, Trends Cell Biol. 10: 290-95 (2000));
(iii) neither the number of protein molecules that can be transferred into a cell by a given translocation domain has been systematically determined, nor the number of Cre molecules in the cell nucleus that is required for efficient recombination;
(iv) the delivery of active proteins requires unfolding- and proper refolding which is unpredictable for a given protein (Bonifaci et al., AIDS 9: 995-1000 1995); and
(v) the mechanism by which protein transduction domains facilitate protein transduction in unknown and several findings have been published that rule out classical receptor-, transporter-, endosome- or endocytosis-mediated processes in the transduction of Ant, TAT and VP22 (G. Eliott, P. O'Hare, Cell 88, 223-233 (1997); D.A. Mann, A.D. Frankel, EMBO. J. 10,

1733-1739 (1991); D. Derossi et al., J. Biol. Chem. 269, 10444-10450 (1994); D. Derossi et al., J. Biol. Chem. 271, 18188-18193 (1996); E. Vives et al., J. Biol. Chem. 272, 16010-16017 (1997)).

Moreover, there was still the need for a generally applicable method where the genetic manipulation can be performed in both, endogenous genes and transgenes.

Summary of the Invention

It was found that site-specific DNA recombinase proteins can be translocated into cells of a living organism when fused to specific protein transduction domains, namely transduction domains being derived from the VP22 protein of HSV or from the TAT protein of HIV. Thus, whenever a gene mutation is desired, recombination is induced upon the injection of the appropriate site-specific recombinase fused to a transduction domain into such a living organism (provided, however, that said organism carries at least one appropriate RRS integrated in the genome).

The present invention thus provides

(1) the use of a fusion protein comprising

(a) a site-specific DNA recombinase domain and

(b) a protein transduction domain (PTD)

for preparing an agent for inducing target gene alterations in a living organism or cell culture, wherein said living organism carries at least one or more recognition sites for said site-specific DNA recombinase integrated in its genome;

(2) a method for inducing gene alterations in a living organism which comprises administering to said living organism a fusion protein comprising a site-specific DNA recombinase domain and a PTD as defined in (1) above, wherein said living organism carries at least one or more

recognition sites for said site-specific DNA recombinase integrated in its genome;

(3) a fusion protein comprising

(a) a site-specific DNA recombinase domain and

(b) a PTD being derived from the VP22 protein of HSV or from the TAT protein of HIV

provided that when the site-specific DNA recombinase domain is wild-type Cre or Flp then the PTD is not the full length VP22 PTD of HSV (i.e., the fusion protein is not identical to the fusion protein of Dalby and Bennett, Invitrogen, Expressions 6.2, page 13 (1999) and of WO 99/60142);

(4) a DNA sequence coding for the fusion protein of (3) above;

(5) a vector comprising the DNA sequence as defined in (4) above;

(6) a host cell transformed with the vector of (5) above and/or comprising the DNA of (4) above;

(7) a method for producing the fusion protein of (1) above which comprises culturing the transformed host cell of (6) above and isolating the fusion protein; and

(8) an injectable composition comprising the fusion protein as defined in (1) or (3) above.

The invention is further illustrated by the appended Figures and is explained in detail below.

Description of the Figures

<u>Fig. 1:</u> Generation of induced mouse mutants using purified fusion proteins.

A: Expression of the fusion protein consisting of the site-specific DNA recombinase (e.g. Cre) and the protein transduction domain (e.g. the HIV derived TAT peptide) in prokaryotic or eukaryotic cells.

B: Extraction and purification of the expressed fusion protein (e.g. as described in Nagahara et al., Nat. Med. 4 (12):1449-52 (1998)).

C: Injection of the purified fusion protein into mice carrying the RRS-flanked target sequence.

D: Analysis of the pattern of induced target gene recombination and the resulting phenotype.

Triangle: RRS.

Fig. 2: Scheme of the bacterial expression vector pT7-TACS (SEQ ID NO:16). The coding region of the 11 amino acid protein transduction domain of HIV TAT protein is fused to the N-terminus of the Cre recombinase protein sequence. The 10-amino-acid strep tag and the protease factor Xa recognition sequence are fused to the C-terminus. The T7 promoter permits expression of TAT-Cre protein in *E. coli*.

Fig. 3: Detection of purified TAT-Cre protein by Coomassie staining and Western blot analysis.

A: Coomassie stained SDS-PAGE gel. Lane 1: 10 kDa ladder (Life Technologies, Cat. No.: 10064-012), 2: 1000 ng BSA, 3: 750 ng BSA, 4: 500 ng BSA, 5: 100 ng BSA, 6: 50 ng BSA, 7: 5 µl TAT-Cre, 8: 1 µl TAT-Cre in Bicine buffer.

B: Western blot analysis using an alkaline phosphatase-conjugated anti-strep tag antibody (IBA, Cat. No: 2-1503-001). Lane 1: MultiMark (Invitrogen, Cat. No.: LC5725), 2: 7 µl TAT-Cre, 3: 5 µl TAT-Cre, 4: 2,5 µl TAT-Cre, 5: 1,25 µl TAT-Cre in Bicine buffer.

Fig. 4: X-Gal staining of M5Pax8 cells treated with TAT-Cre protein. M5Pax8 fibroblasts where treated for 18 h with 3,5 (A), 6,9 (B) and 13,8 µg/ml TAT-Cre protein (C) in serum-free medium. Four days after treatment, cells were fixed and stained with X-Gal.

Fig. 5: Measurement of ß-galactosidase activity in cell lysates. M5Pax8 fibroblasts where treated for 18 h with increasing concentrations of TAT-Cre, as indicated, or transiently transfected with either expression vectors

for Cre (pCMV-I-Cre-pA, see SEQ ID NO:29) or ß-galactosidase (pCMV-I-ß-pA, see SEQ ID NO:30). Four days after treatment, cells were lysed and the ß-galactosidase activities were determined.

<u>Fig. 6:</u> PCR detection of TAT-Cre mediated recombination in mice.
A: PCR-analysis of genomic DNA from duodenum (lane 2), liver (3), kidney (4), spleen (5), muscle (6), lung (7), tail (8) and brain (9) of a *pln13* mouse treated three times with intraperitoneal injections of 75 µg TAT Cre protein at two-day-intervals. Deletion of the loxP-flanked DNA segment is indicated by the presence of the about 400 bp fragment. Lane 1: 1-kb-ladder (Life Technologies).
B: PCR strategy to detect Cre-mediated deletion of the loxP-flanked DNA segment. Arrows indicate the positions of the primers.
C: PCR-analysis of genomic DNA from spleen of a *pln13* mouse treated three times with intraperitoneal injections of 75 µg TAT Cre protein at two-day-intervals (lane 4). To confirm the presence of the BamH I restriction site, the PCR product was digested with BamH I which produces two diagnostic fragments of about 190 and about 210 bp (5). As a control, tail DNA from untreated mice carrying the loxP-flanked (lane 2) and the detected pln13 allele (3) was subjected to PCR amplification. Lane 1: 100 bp ladder (Life Technologies), lane 6: 1 kb ladder (Life Technologies).

<u>Fig. 7:</u> Scheme of the bacterial expression vectors pT7-VPCS (SEQ ID NO:17) and pCRT7-ΔVPCS (SEQ ID NO:15). The coding region of the 301 amino acid protein transduction domain of HSV VP22 protein (A) or the truncated 143 amino acid ΔVP22 domain (B) is fused to the N-terminus of the Cre recombinase protein sequence. The 10-amino-acid strep tag and the protease factor Xa recognition sequence are fused to the C-terminus. The T7 promoter allows the expression of VP22-Cre and ΔVP22-Cre fusion proteins in *E. coli*. The sequence in pCRT7-ΔVPCS encoding the 15 amino

acid N-terminal leader sequence is used for enhanced protein stability (Invitrogen).

Fig. 8: Detection of the purified VP22-Cre and ΔVP22-Cre fusion proteins by Coomassie staining and Western blot analysis.
A: Detection of VP22-Cre protein in a Coomassie-stained SDS-PAGE gel. Lane 1: 10 kDa ladder, 2: 1000 ng BSA, 3: 500 ng BSA, 4: 100 ng BSA, 5: inclusion body protein extract before chromatography, 6: unbound protein, 7: fraction 17, 8: fraction 18, 9: fraction 19, 10: fraction 20. The position of the 75 kDa VP22-Cre protein is indicated by the arrow head.
B: Detection of VP22-Cre protein by Western blot analysis using an alkaline phosphatase-conjugated anti-strep tag antibody (IBA, Cat. No.: 2-1503-001). Lane 1: MultiMark (Invitrogen), 2: inclusion body protein extract before chromatography, 3: unbound protein, 4: fraction 10, 5: fraction 11, 5: fraction 16, 6: fraction 17, 7: fraction 18, 8: fraction 19, 9: fraction 19, 10: fraction 20.
C: Detection of ΔVP22-Cre protein in a Coomassie-stained SDS-PAGE gel. Lane 1: 10 kDa ladder, 2: inclusion body protein extract before chromatography, 3: unbound protein, 4: fraction 1, 5: fraction 8, 6: fraction 9, 7: fraction 15, 8: 100 ng BSA, 9: 500 ng BSA, 10: 1000 ng BSA. The position of the 60 kDa ΔVP22-Cre protein is indicated by the arrow head.
D: Detection of ΔVP22-Cre protein by Western blot analysis using a alkaline phosphatase-conjugated anti-strep tag antibody (IBA, Cat. No.: 2-1503-001). Lane 1: MultiMark (Invitrogen), 2: inclusion body protein extract before chromatography, 3: unbound protein, 4: fraction 4, 5: fraction 8, 6: fraction 10, 7: fraction 12, 8: soluble protein extract before chromatography, 9: unbound protein, 10: fraction 7.

Fig. 9: X-Gal staining of M5Pax8 cells treated with VP22-Cre and ΔVP22-Cre fusion proteins. M5Pax8 fibroblasts where treated for 18 h with either

Bicine buffer (A), 0.5 µg/ml VP22-Cre (B) or 3.75 g/ml ΔVP22-Cre (C) in serum-free medium. Four days after treatment, cells were fixed and stained with X-Gal.

Fig. 10: Measurement of ß-galactosidase activity in cell lysates. M5Pax8 fibroblasts where treated for 18 h with VP22-Cre, ΔVP22-Cre or Bicine buffer alone, as indicated or transiently transfected with expression vectors for Cre (pCMV-I-Cre-pA, see SEQ ID NO:29) or ß-galactosidase (pCMV-I-ß-pA, see SEQ ID NO:30). Four days after treatment, cells were lysed and the ß-galactosidase activities were determined.

Fig. 11: PCR detection of Cre mediated recombination in cells treated with VP22-Cre and ΔVP22-Cre fusion proteins shown in SEQ ID NOs: 21 and 14, respectively).

A: PCR-analysis of genomic DNA isolated from M5Pax8 fibroblasts. Cells were transiently transfected with a Cre expression vector (lane 2) or treated for 18 h with either buffer alone (lane 3), 7.5 µg/ml VP22-Cre (4, 5) or 15 µg/ml ΔVP22-Cre (6, 7) in serum-free medium. Four days after treatment, genomic DNA was extracted and subjected to PCR amplification. Deletion of the loxP-flanked DNA segment is indicated by the presence of the 226 bp DNA fragment. To confirm the presence of the Nco I restriction site in the recombined allele, the PCR products were digested with Nco I which produces two diagnostic fragments of 85bp and 141bp (lanes 5 and 7). Lane 1: 100 bp ladder (Life Technologies), lane 8: 1 kb ladder (Life Technologies).

B: PCR strategy to detect Cre-mediated deletion of the loxP-flanked DNA segment. Arrows indicate the positions of the primers.

Detailed Description of the Invention

The expression "target sequences" according to the present invention means all kind of sequences which may be mutated (viz. deleted,

translocated, integrated and/or inverted) by the action of the recombinase. The number of RRS in the target sequence depends on the kind of mutation to be performed by the recombinase. For most of the mutations (especially for deletions and invertions) two RRS are required which are flanking the sequence to be mutated (deleted or inverted). For some kinds of integrations only one RRS may be necessary within the target sequence.

The "living organisms" according to the present invention are multi-cell organisms and can be vertebrates such as mammals (e.g., rodents such as mice or rats) or non-mammals (e.g., fish) or can be invertebrates such as insects or worms, or can be plants (higher plants, algi or fungi). Most preferred living organisms are mice and fish.

"Cell culture" according to the present invention include cells isolated from the above defined living organism and cultured *in vitro.* These cells can be transformed (immortalized) or untransformed (directly derived from the living organism; primary cell culture).

The site-specific DNA recombinase domain within the fusion protein of the invention of the present application is preferably selected from a recombinase protein derived from Cre, Flp, ϕC31 recombinase (Thorpe and Smith, Proc. Natl. Acad. Sci, USA, vol. 95, 5505-5510 (1998)), $\gamma\delta$ resolvase (Schwickardi and Dröge, FEBS letters 471:147-150 (2000) and R recombinase (Araki et al., J. Mol. Biol., 182, 191-203 (1985)). The preferred recombinases are Cre and mutants thereof (preferably the Cre variant of aa 15 to 357 of SEQ ID NO: 2 or aa 325-667 of SEQ ID NO: 6) and Flp and variants thereof including Flpe (preferably the Flp variant of aa 15 to 437 of SEQ ID NO: 4 or aa 325 to 747 of SEQ ID NO: 8).

The protein transduction domain according to the present invention includes, but is not limited to, the PTDs mentioned in Background of the Invention. The PTD preferably is derived from the VP22 protein of HSV or from the TAT protein of HIV. Suitable TAT proteins include, but are not limited to, proteins comprising (i) the amino acid sequence shown in SEQ ID NO: 10 and mutant thereof such as
(ii) proteins comprising the amino acid
AGRKKRRQRRR (SEQ ID NO:22)
YARKARRQARR (SEQ ID NO:23)
YARAAARQARA (SEQ ID NO:24)
YARAARRAARR (SEQ ID NO:25)
YARAARRAARA (SEQ ID NO:26)
YARRRRRRRRR (SEQ ID NO:27)
YAAARRRRRRR (SEQ ID NO:28)
as known from WO 99/29721. Preferred are transduction domains consisting of the TAT proteins (i) and (ii) above.

Suitable VP22 proteins include, but are not limited to, the wild-type VP22 protein, i.e., a protein comprising amino acids 1 to 302 of SEQ ID No:21, and truncated forms thereof. Truncated VP22 proteins in accordance with the present invention can be those lacking 1 to 158 amino acid residues at their N-terminal end. The most preferred VP22 protein is the truncated VP22 PTD comprising amino acid residues 16 to 157 of SEQ ID NO:14.

The fusion of the two domains of the fusion protein can occur at any possible position, i.e., the protein transduction domain can be fused to the N- or C-terminal of the site-specific DNA recombinase or can be fused to active sites within the site-specific DNA recombinase. Preferably the protein transfusion domain is fused to the N-terminal of the site-specific DNA recombinase domain.

The protein transduction domain can be fused to the site-specific DNA recombinase either through a direct chemical bond or through a linker molecule. Such linker molecule can be any bivalent chemical structure capable of linking the two domains. The preferred linker molecule according to the present invention is a short peptide, e.g., having 1 to 20, preferably 1 to 10, amino acid residues. Specifically preferred short peptides are essentially consisting of Gly, Ala and/or Leu.

The fusion protein of the invention of the present application may further comprise other functional sequences such as secretion conferring signals, nuclear localisation signals and/or signals conferring protein stabilisation.

In case the fusion protein comprises a protein transduction domain derived from the TAT protein of HIV, the DNA sequence coding for said fusion protein preferably comprises the sequence
5' TAC GGC CGC AAG AAG CGC CGC CAA CGC CGC CGC 3'.

Such a preferred DNA sequence is for instance shown in SEQ ID NO: 11. In said sequence the 3' terminal codon ggc codes for the linker Gly. The DNA sequence of a suitable recombinase may be directly attached to said codon ggc.

The fusion protein can be obtained by the following steps:

1. Fusion of the recombinase coding region (e.g. encoding Cre: see amino acids 15 to 357 of SEQ ID NO: 2) with the sequence conferring protein translocation (e.g. the sequence encoding the TAT peptide YGRKKRRQRRR, SEQ ID NO: 10) using standard cloning protocols (Maniatis et al., Cold Spring Harbor Laboratory, New York (1989)) or chemical synthesis.

2. Generation of a construct for the expression of the fusion protein in prokaryotic or eukaryotic cells, e.g. in E. coli DH5a (Hanahan, J. Mol. Biol.;166(4):557-80 (1983)) using the QIAexpress pQE vector (Qiagen, Hilden).
3. Expression of the above mentioned fusion protein in prokaryotic or eukaryotic cells, e.g. in E. coli DH5a (Hanahan, 1983)
4. Extraction and purification of the above mentioned fusion protein e.g. as described in Nagahara et al., Nat. Med., 4(12):1449-52 (1998).

In an experiment it was shown that TAT-mediated delivery of active Cre protein works with sufficient efficacy to facilitate inducible gene targeting both in cell lines and living organisms. In this experiment a vector for the expression of a TAT-Cre fusion protein in E. coli was constructed, TAT-Cre protein was expressed in E. coli and purified from bacterial lysates. To test the activity of the TAT-Cre protein *in vitro*, a reporter cell line that contains a loxP-containing reporter construct was used. This reporter, when recombined by Cre recombinase, allows the expression of a ß-galacosidase gene. Further, a transgenic mouse strain carrying a loxP-flanked target was used to invest the activity of the TAT-Cre protein *in vivo*.

In a second experiment it was shown that VP22-mediated delivery of active Cre protein works with sufficient efficacy to facilitate inducible gene targeting. In this experiment Bacterial expression vectors were constructed for the production of VP22-Cre fusion proteins in E. coli. The activity of purified VP22-Cre proteins were tested using a reporter fibroblast cell line containing a loxP-flanked reporter construct.

Thus, the injection of the purified fusion protein of the present invention into a living organism (e.g., a mouse) carrying a gene comprising the RRS-flanked target sequence (e.g., in an amount of 1 to 200, preferably 5

to 50 µg per g body weight). To demonstrate the feasibility of the invention, a reporter mouse strain carrying an RRS-flanked cassette was used (Thorey et al., Mol. Cell Biol., 18(10):6164 (1998)).

Analysis is achieved by determining the pattern of induced target gene recombination (e.g. through PCR analysis, Southern blot analysis or X-Gal staining on tissue sections; Maniatis et al., 1989; Gossler and Zachgo, Joyner AL (Ed.), Oxford University Press, Oxford, New York (1993)).

The procedure's advantages over current technology are as follows:

(i) The absence of background recombination before administration of the fusion protein.

(ii) The reduction of time and resources which are necessary to combine the recombinase transgene and two copies of the RRS-flanked target gene by conventional breeding.

In experiments it was shown the following: (a) With a suitable vector for the expression of a TAT-Cre fusion protein, a TAT-Cre fusion protein was expressed in *E. coli* and purified from bacterial lysates.
(b) A reporter cell line containing a loxP-containing reporter construct was used to test the activity of the TAT-Cre protein *in vitro*. This reporter, when recombined by Cre recombinase, allows the expression of a ß-galacosidase gene.
(c) A transgenic mouse strain carrying a loxP-flanked target was used to invest the activity of the TAT-Cre protein *in vivo*.
These experiments demonstrate that TAT-mediated delivery of active Cre protein works with sufficient efficacy to facilitate inducible gene targeting both in cell lines and living organisms.

Furthermore, bacterial expression vectors were constructed for the production of VP22-Cre fusion proteins in E. coli. The activity of purified VP22-Cre proteins were tested using a reporter fibroblast cell line containing a loxP-flanked reporter construct. These experiments demonstrate that VP22-mediated delivery of active Cre protein works with sufficient efficacy to facilitate inducible gene targeting.

The invention is further illustrated by the following, non-limitative examples.

Examples

Materials and Methods

Construction of pT7-TACS: The TAT-Cre coding region was generated by PCR using Advantage-HF PCR Kit (Clontech), 20 pmol of the primers TATcre sense (5′-atg cca tgg gct acg gcc gca aga agc gcc gcc aac gcc gcc gcg gca tgt cca att tac tga ccg tac acc-3′; SEQ ID NO:31) and TATcre antisense (5′-ttt cgg atc cgc cgc ata acc agt g-3′; SEQ ID NO:32) and 10 ng pCMV-I-Cre-pA (see SEQ ID NO:29) as template. The PCR reaction was performed using the following cycle profile: 2′ 94 °C, 4 x (30″ 94 °C min, 30″ 50 °C, 1′ 72 °C), 12 x (30″ 94 °C min, 30″ 55 °C, 1′ 72 °C) and 10′ 72 °C. The resulting PCR fragment was digested with Nco I and BamH I, treated with Klenow enzyme and ligated into the plasmid pBSII KS+ which had been opened with restriction enzyme BamH I, treated with Klenow and dephosphorylated with calf intestinal phosphatase. The resulting plasmid pBS TAT-5′cre was verified by DNA sequencing. The Plasmid pCMV-I-Cre-pA (SEQ ID NO:29) was digested with Age I and Sal I which released a 1,036 kb fragment containing the 3′ part of the Cre coding region. This fragment was ligated into the plasmid pBS TAT-5′cre which had been opened with Age I and Sal I.

10 ng pBS-TATCre was subjected to PCR amplification using 20 pmol of primers FPA001 (5'-tat atc tag acc atg ggc tac ggc cgc aag aag c-3'; SEQ ID NO:33) and FPA002 (5'-gct acc acg acc ttc gat acc atc gcc atc ttc cag cag gcg c-3'; SEQ ID NO:34). PCR was performed using 2,5 U Platinum Pfx DNA polymerase (Gibco BRL) and 2 x Enhancer Solution (Gibco BRL) according to the manufacturers protocol. The following cycle profile was used: 2' 94 °C, 25 x (30" 94 °C min, 15" 54,6 °C, 2'30" 68 °C). The amplified PCR fragment was purified using GFX columns (Amersham Pharmacia), digested with Xba I and ligated into the plasmid pASK57 (Skerra and Arne, Gene 151: 131-135 (1994)) which had been opened with restriction enzymes Xba I and Eco 47 III and dephosphorylated with calf intestinal phosphatase. The resulting plasmid pASK75-TACS was digested with restriction enzymes Nco I and Hind III which released a 1,1 kb fragment. The fragment was subsequently ligated into the plasmid pT7-7 (Studier and Moffatt, J. Mol. Biol. 189: 113-130 (1986)) which had been opened with restriction enzymes Nco I and Hind III and dephosphorylated with calf intestinal phosphatase resulting in the plasmid pT7-TACS (SEQ ID NO:16).

Construction of pT7-VPCS: The Cre coding region was generated by PCR using Advantage-HF PCR Kit (Clontech), 20 pmol of the primers VP22cre sense (5'-taa cta gcg gcc gca tgt cca att tac tga ccg tac ac-3'; SEQ ID NO:35) and VP22cre antisense (5'-tcg agc ggc cgc cat cgc cat ctt cca gca ggc g-3'; SEQ ID NO:36) and 10 ng pgkcre-pA (SEQ ID NO:40) as template. The PCR reaction was performed using the following cycle profile: 2' 94 °C, 5 x (30" 94 °C, 30" 50 °C, 2' 72 °C), 15 x (30" 94 °C, 30" 55 °C, 2' 72 °C) and 10' 72 °C. The resulting PCR fragment was digested with Not I and ligated into the plasmid pVP22/Myc-His (Invitrogen), which had been opened with restriction enzyme NotI, dephosphorylated with calf intestinal phosphatase. The resulting plasmid pVP22-cre myc/His was verified by DNA sequencing.

10 ng pVP22-cre myc/His was subjected to PCR amplification using 20 pmol of primers FPA004 (5′-tat atc tag aca tat gac ctc tcg ccg ctc cg-3′; SEQ ID NO:37) and FPA002 (SEQ ID NO:34). PCR was performed using 2,5 U Platinum Pfx DNA polymerase (Gibco BRL) and 2 x Enhancer Solution (Gibco BRL) according to the manufacturers protocol. The following cycle profile was used: 2′ 94 °C, 25 x (30″ 94 °C min, 15″ 54,6 °C, 2′30″ 68 °C). The amplified PCR fragment was purified using GFX columns (Amersham Pharmacia), digested with Xba I and ligated into the plasmid pASK57 (Skerra and Arne, Gene 151: 131-135 (1994)) which had been opened with restriction enzymes Xba I and Eco 47 III and dephosphorylated with calf intestinal phosphatase. The resulting plasmid pASK75-VPCS was digested with restriction enzymes Nde I and Hind III which released a 2,0 kb fragment. The fragment was subsequently ligated into the plasmid pT7-7 (Studier and Moffatt, J. Mol. Biol. 189: 113-130 (1986)) which had been opened with restriction enzymes Nde I and Hind III and dephosphorylated with calf intestinal phosphatase resulting in the plasmid pT7-VPCS (SEQ ID NO:17).

<u>Construction of pCRT7-ΔVPCS:</u> The ΔVP22-Cre coding region was generated by PCR using Platinum Pfx DNA polymerase (Life Technologies), 20 pmol of the primers FPA007 (5'-ttc cga aga cga cga aac acc-3'; SEQ ID NO:38) and FPA008 (5'-tat att cga agc tta tta acc acc gaa ctg cg-3'; SEQ ID NO:39) and 30 ng pT7-VPCS (SEQ ID NO:17) as template. The PCR reaction was performed using the following cycle profile: 2' 94 °C, 25 x (30" 94 °C, 30" 61 °C, 2'30″ 68 °C) and 7' 68 °C. The resulting 1,8 kb PCR fragment was digested with Nco I and Sfu I and ligated into the plasmid pCRT7/VP22-1 (Invitrogen), which had been opened with restriction enzymes Nco I and Sfu I, and dephosphorylated with calf intestinal phosphatase. The resulting plasmid pCRT7-ΔVPCS (SEQ ID NO:15) was verified by DNA sequencing.

<u>Expression of the fusion proteins in E. coli:</u> E. coli BL21(DE3)-RIL cells (Stratagene) were transformed with pT7-TACS and grown on LB agar plates containing 100 µg/ml ampicillin. E. coli BL21(DE3)-RP cells (Stratagene) were transformed with pT7-VPCS and grown on LB agar plates containing 100 µg/ml ampicillin. E. coli BL21(DE3)-pLysS (Invitrogen) were transformed with pCRT7-ΔVPCS and grown on LB agar plates containing 25 µg/ml kanamycine and 34 µg/ml chloramphenicol. Single colonies were isolated and used to prepare glycerol stocks. Eight 5ml LB (Lura Bertani) aliquots containing antibiotics were inoculated with stabs from the glycerol stocks and grown overnight at 37°C with shaking. Two 5ml overnight cultures were each used to inoculate one of four 1L LB aliquots containing antibiotics and grown at 37°C with shaking. Growth rate was monitored by spectrophotometry at 578nm. When the cultures had obtained an OD_{578} = 0,5 expression of the fusion proteins were induced by the addition of 0,5 mM Isopropyl-ß-D-1-thiogalactopyranosid (IPTG). Two hours after induction cells were harvested by centrifugation at 12000xg and the pellet rapidly frozen in liquid nitrogen and stored immediately at −80°C.

<u>Purification of the fusion proteins from bacterial lysates:</u> Each 10g cell pellet was resuspended on ice in 30ml Bicine buffer (50mM Bicine, pH 8,5) including one protease inhibitor tablet (Complete, Roche). Cells were lysed through threefold treatment (1500psi, 5 minutes) with the cell disruption bomb (Parr Instrument). 30ml of Benzonase (10000U, Merck) was added and cell extracts were incubated for 30 minutes at 4°C. Cell extracts were then centrifuged at 12,000xg (4°C). The pellet was redissolved in 8M urea, 50mM Bicine, 100mM DTT, pH 8,5 by incubation for 16 hours at 4°C. Protein extract was centrifuged at 31000xg and supernatant harvested. Protein extract was diluted in an equal volume of Chromatography buffer A (50mM Bicine, pH 8,5). PH was adjusted to pH

8,5 and the extract was filtered through a 0,45µm filter (Millipore). FPLC (Akta Explorer, Amersham Pharmacia) was performed using a cation exchange column (Sepharose SP, Column body HR_5/5 (0.5 x 5cm), column volume (CV) 1ml, linear flow 300cm/hour, Amersham Pharmacia). After addition of sample to FPLC column, buffer was exchanged with Chromatography buffer A at 10 CV.

TAT-Cre and VP22-Cre fusion proteins were eluted from the column by gradient elution using chromatography buffer B (50mM Bicine, 1M NaCl, pH 8,5) using the following profile: 0 - 50 % buffer B, 0 CV; 50 % buffer B, 10 CV; 50 - 100 % buffer B (linear gradient), 20 CV; 100 % buffer B, 10 CV. ΔVP22-Cre protein was eluted from the column by gradient elution using the following profile: 0 - 10 % buffer B, 0 CV; 10 % buffer B, 10 CV; 10 – 30 % buffer B, 0 CV; 30 % buffer B, 10 CV; 30 – 100 % buffer B, 0 CV; 100 % buffer B, 10 CV. Three 1,5ml fractions each containing purified fusion proteins were collected. Purity and concentration of protein fractions were determined by Coomassie blue stained SDS-PAGE gels and Western blot analysis using dilutions of BSA standard solutions. In addition protein content was determined using a Bradford assay (Coomassie Plus protein assay, Pierce).

<u>SDS-PAGE and Western blot analysis:</u> SDS-PAGE and Coomassie staining was performed according to standard protocols (Maniatis et al., Cold Spring Harbor Laboratory, New York (1989)) using 4 - 12 % gradient SDS-polyacrylamide gels (NuPAGE, Invitrogen, cat. no.: NPO321). Western blot analysis was performed using a Semi-Try Blotting Chamber (Biorad) and nitrocellulose membranes (0,2 µm; Schleicher & Schuell) according to the manufacturers protocols. The fusion proteins were detected by using an alkaline phosphatase-conjugated anti-strep tag antibody (IBA, Cat. No.: 2-1503-001) according to the manufacturers protocol.

Generation of the M5Pax8 Cre reporter cell line: The SV40-transformed murine embryonic fibroblast line MEF5/5 (Schwenk et al., Nucl Acids Res 26(6), 1427-32 (1998)) was transfected with the vector pPGKpaX1 (Kellendonk et al., Nucl. Acids Res. 24, 1404-11 (1996)). 10^6 MEF5/5 cells were electroporated with 20 µg pPGKpaX1 plasmid DNA linearised with Sca I and plated into 48-well-plates. The cells were cultured in DMEM/Glutamax medium (Life Technologies) supplemented with 10 % fetal calf serum at 37°C, 10 % CO_2 in humid atmosphere. Two days after transfection the medium was supplemented with 5 µg/ml puromycine (Calbiochem) for the selection of stable integrants. 14 puromycine-resistant clones were expanded and tested by transien transfection with the Cre expression vector pPGK-Cre-pA (SEQ ID NO: 40). In two out of the 14 puromycine-resistant clones, the expression of ß-galactosidase could be detected by staining with X-Gal. One of these clones, M5Pax8, was used as Cre reporter cell line.

Transfection and measurement of ß-galactosidase activity: Fibroblasts (10^6 cells per 24 well plate (Falcon)) were transfected with 25 ng pCMV-I-Cre-pA (see SEQ ID NO:29) or pCMV-I-ß-pA (see SEQ ID NO:30) plasmids using the FuGene transfection reagent (Roche Diagnostics). After 2 days the cells were lysed and the ß-galactosidase activities were determined with the ß-galactosidase reporter gene assay (Roche Diagnostics) according to the manufacturers guidelines using a Lumistar luminometer (MWG).

Histochemical detection of ß-galactosidase activity: To quantitate ß-galactosidase expression, fibroblast cells were washed once with phosphate buffered saline (PBS), and the cells were fixed for 5 minutes at room temperature in a solution of 4% formaldehyde in PBS. Next, the cells were washed twice with PBS and finally incubated in staining solution for 24 hours at 37°C (staining solution: 5 mM K3(Fe(CN)6), 5mM

K4(Fe(CN)6), 2mM MgCl2, 1mg/ml X-Gal (BioMol) in PBS). Blue stained, ß-galactosidase positive cells were detected and distinguished from negative (transparent) cells in a cell culture binocular microscope under 200x magnification. For each determination a minimum of 200 cells was counted.

PCR detection of Cre-mediated recombination: Genomic DNA extracted from tissue samples was subjected to PCR using Taq-polymerase (Gibco BRL Cat. No. 10342-020) using 20 pmol of each primer (sense: 5`-CAT CTC CGG GCC TTT CGA CCT G - 3', antisense: 5' –GCG ATC GGT GCG GGC CTC TTC - 3'; SEQ ID Nos: 41 and 42, respectively). PCR was performed using the following cycle profile: 2' 94°C, 35 x (30" 94°C, 30" 55 °C, 1' 72 °C), 10 min 72 °C. PCR products were separated on a 1,2 % agarose gel.

Example 1

The vector pT7-TACS (SEQ ID NO:16) was constructed for the expression of a TAT-Cre fusion protein in E. coli. The plasmid contains the coding region of the 11 amino acid protein transduction domain of the wild-type HIV TAT protein (Green and Loewenstein, Cell, 55(6):1179-88 (1988); Frankel and Pabo, Cell, 55(6): 1189-93 (1988); SEQ ID NO:10) fused to the N-terminus of Cre recombinase protein sequence. The 10-amino-acid strep tag at the C-terminus allows the detection and purification of the fusion protein using specific antibodies (Schmidt and Skerra, J. Chromatogr A 676: 337-345 (1994)). The protease factor Xa recognition site (Ile-Glu-Gly-Arg) permits the removal of the strep tag by proteolytic cleavage. The estimated molecular weight of the TAT-Cre fusion protein is 42 kDa. A scheme of the TAT-Cre expression vector is depicted in figure 2. For the expression of TAT-Cre, the E. coli strain BL21(DE3)-RIL (Stratagene) was used. This strain carries an IPTG-inducible T7 polymerase gene and additional copies of the tRNA genes for the 'rare

codons' argU, ileY and leuW.

E. coli BL21(DE3)-RIL cells were transformed with pT7-TACS and grown in LB medium containing 100 µg/ml ampicillin. The expression of the 40 kDa TAT-Cre fusion protein could be strongly induced by the addition of 0,5 mM IPTG to the culture medium. Analysis of protein lysates revealed that approximately 50 % of TAT-Cre protein accumulated as insoluble inclusion bodies. The inclusion bodies where extracted and dissolved in 8 M urea. TAT-Cre was subsequently purified from this fraction using ion exchange chromatography. The quantity and purity of TAT-Cre protein was determined using Coomassie stained SDS-PAGE gels and Western blot analysis (figure 3). The purification process yielded TAT-Cre protein extracts of 64 % purity and a concentration of 100 µg/ml.

To analyse the ability of the purified TAT-Cre protein to transduce into cultured cells, we used the fibroblast cell line M5Pax8 (R. Kühn, unpublished) that contains a loxP-containing reporter construct. This reporter, when recombined by Cre recombinase, allows the expression of a ß-galacosidase gene (Buchholz et al, Nucleic Acids Res. 24, 4256-4262, 1996). Cells were cultured for 18 h with increasing concentrations of TAT-Cre protein in serum-free medium and analysed 4 days later for ß-Galacosidase activity. Staining with X-Gal showed that > 50 % of the cells treated with 13,8 µg/ml TAT-Cre protein expressed ß-galactosidase indicating recombination of the loxP-flanked reporter construct had occurred (figure 4). Measurement of ß-galactosidase activity in cell lysates revealed an up to 30-fold higher level of ß-galactosidase activity in comparison to cells which had been transiently transfected with an eukaryotic Cre expression vector (figure 5).

To investigate the activity of TAT-Cre protein in a living organism, we used a transgenic mouse strain carrying a loxP-flanked target for Cre-mediated recombination (Thorey et al., 1998, Mol. Cell. Biol. 18: 3081 – 3088). Mice where treated three times with intraperitoneal injections of 75 µg TAT Cre protein at two-day-intervals and analysed 2 days later. Genomic DNA was

isolated from a variety of organs and subjected to PCR amplification which specifically amplifies a 400 bp fragment of the recombined allele. The deleted allele could be detected in multiple tissues from treated mice indicating TAT-Cre-mediated recombination in these organs (figure 6). This experiments demonstrates that TAT-mediated delivery of active Cre protein works with sufficient efficacy to facilitate inducible gene targeting in cell lines and in living organisms.

Example 2

The vectors pT7-VPCS (SEQ ID NO:17) and pCRT7-ΔVPCS (SEQ ID NO:15) were constructed for the expression of VP22-Cre and ΔVP22-Cre fusion proteins in E. coli. The VP22-Cre gene of pT7-VPCS contains the full length protein translocation domain of the HSV VP22 protein (Elliott and O'Hare, Cell, 88(2): 223-33 (1987), whereas the ΔVP22-Cre gene of pCRT7-ΔVPCS contains a truncated VP22 protein transduction domain (amino acids 159 – 301; Invitrogen; aa 16-157 of SEQ ID NO:14) fused to the N-terminus of Cre recombinase protein sequence. A 10-amino-acid strep tag at the C-terminus of Cre protein sequence allows the detection and purification of the fusion proteins using specific antibodies (Schmidt and Skerra, J. Chromatogr A 676: 337-345 (1994)). The protease factor Xa recognition site permits the removal of the Strep tag by proteolytic cleavage. The estimated molecular weight is 75 kDa for VP22-Cre protein and 60 kDa for ΔVP22-Cre protein. A scheme of the vectors pT7-VPCS and pCRT7-ΔVPCS is depicted in figure 7.

E. coli BL21(DE3)-RIP cells (Stratagene) were transformed with pT7-VPCS and cultured in LB medium containing 100 µg/ml ampicillin. E. coli BL21(DE3)-pLysS cells (Stratagene) were transformed with pCRT7-ΔVPCS and cultured in LB medium containing 25 µg/ml kanamycine and 34 µg/ml chloramphenicol. Expression of the VP22-Cre and ΔVP22-Cre fusion proteins could be induced by the addition of 0,5 mM IPTG to the culture medium. Analysis of protein extracts using Coomassie staining and

Western blotting of SDS-PAGE gels revealed that 50 - 60 % of VP22-Cre and ΔVP22-Cre proteins accumulated as insoluble inclusion bodies. The inclusion bodies where extracted and dissolved in 8 M urea. VP22-Cre and ΔVP22-Cre fusion proteins were subsequently purified using ion exchange chromatography. The quantity and purity of the isolated VP22-Cre and Δ VP22-Cre fusion proteins was determined using Coomassie stained SDS-PAGE gels and Western blot analysis (figure 8).
To analyse the ability of the purified fusion proteins to transduce into cultured cells, we used the fibroblast cell line M5Pax8 that contains a loxP-containing reporter construct. When recombined by Cre recombinase, the reporter allows the expression of a ß-galacosidase gene (Buchholz et al, Nucleic Acids Res. 24, 4256-4262, 1996). The cells where cultured for 18 h with increasing concentrations of VP22-Cre and ΔVP22-Cre in serum-free medium and analysed 4 days later for ß-Galacosidase activity. Staining with X-Gal showed ~2 % blue cells in the cultures treated with up to 15 µg/ml ΔVP22-Cre indicating recombination of the loxP-flanked reporter construct had occurred. In contrast, cell cultures treated with up to 0,5 µg/ml VP22-Cre did not show any X-gal staining (figure 9). Measurement of cell lysates revealed a strong increase of ß-galactosidase activity upon Δ VP22-Cre treatment when compared to untreated cells (figure 10). Genomic DNA was isolated fand subjected to PCR amplification that specifically amplifies a 250 bp fragment of the recombined allele. The deleted allele could be detected in cells treated with both VP22-Cre and Δ VP22-Cre fusion proteins (figure 11).

This experiment demonstrates that VP22-mediated delivery of active Cre protein works with sufficient efficacy to facilitate inducible gene targeting.

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Ala Ala Glu Leu Thr Tyr Leu Cys Trp Met Ile Thr His Asn Gly Thr
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gca atc aag aga gcc aca ttc atg agc tat aat act atc ata agc aat     240
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Ser Leu Ser Phe Asp Ile Val Asn Lys Ser Leu Gln Phe Lys Tyr Lys
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Ala Trp Glu Phe Thr Ile Ile Pro Tyr Asn Gly Gln Lys His Gln Ser
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gat atc act gat att gta agt agt ttg caa tta cag ttc gaa tca tcg     432
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Leu Leu Ser Glu Gly Glu Ser Ile Trp Glu Ile Thr Glu Lys Ile Leu
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aat tcg ttt gag tat acc tcg aga ttt aca aaa aca aaa act tta tac     576
Asn Ser Phe Glu Tyr Thr Ser Arg Phe Thr Lys Thr Lys Thr Leu Tyr
            180                 185                 190

caa ttc ctc ttc cta gct act ttc atc aat tgt gga aga ttc agc gat     624
Gln Phe Leu Phe Leu Ala Thr Phe Ile Asn Cys Gly Arg Phe Ser Asp
        195                 200                 205

att aag aac gtt gat ccg aaa tca ttt aaa tta gtc caa aat aag tat     672
Ile Lys Asn Val Asp Pro Lys Ser Phe Lys Leu Val Gln Asn Lys Tyr
    210                 215                 220

ctg gga gta ata atc cag tgt tta gtg aca gag aca aag aca agc gtt     720
Leu Gly Val Ile Ile Gln Cys Leu Val Thr Glu Thr Lys Thr Ser Val
225                 230                 235                 240

agt agg cac ata tac ttc ttt agc gca agg ggt agg atc gat cca ctt     768
Ser Arg His Ile Tyr Phe Phe Ser Ala Arg Gly Arg Ile Asp Pro Leu
                245                 250                 255

gta tat ttg gat gaa ttt ttg agg aat tct gaa cca gtc cta aaa cga     816
Val Tyr Leu Asp Glu Phe Leu Arg Asn Ser Glu Pro Val Leu Lys Arg
            260                 265                 270

gta aat agg acc ggc aat tct tca agc aac aaa cag gaa tac caa tta     864
Val Asn Arg Thr Gly Asn Ser Ser Ser Asn Lys Gln Glu Tyr Gln Leu
        275                 280                 285

tta aaa gat aac tta gtc aga tcg tac aac aag gct ttg aag aaa aat     912
Leu Lys Asp Asn Leu Val Arg Ser Tyr Asn Lys Ala Leu Lys Lys Asn
    290                 295                 300

gcg cct tat cca atc ttt gct ata aag aat ggc cca aaa tct cac att     960
Ala Pro Tyr Pro Ile Phe Ala Ile Lys Asn Gly Pro Lys Ser His Ile
305                 310                 315                 320

gga aga cat ttg atg acc tca ttt ctg tca atg aag ggc cta acg gag    1008
Gly Arg His Leu Met Thr Ser Phe Leu Ser Met Lys Gly Leu Thr Glu
                325                 330                 335

ttg act aat gtt gtg gga aat tgg agc gat aag cgt gct tct gcc gtg    1056
Leu Thr Asn Val Val Gly Asn Trp Ser Asp Lys Arg Ala Ser Ala Val
            340                 345                 350

gcc agg aca acg tat act cat cag ata aca gca ata cct gat cac tac    1104
Ala Arg Thr Thr Tyr Thr His Gln Ile Thr Ala Ile Pro Asp His Tyr
        355                 360                 365

ttc gca cta gtt tct cgg tac tat gca tat gat cca ata tca aag gaa    1152
Phe Ala Leu Val Ser Arg Tyr Tyr Ala Tyr Asp Pro Ile Ser Lys Glu
    370                 375                 380

atg ata gca ttg aag gat gag act aat cca att gag gag tgg cag cat    1200
Met Ile Ala Leu Lys Asp Glu Thr Asn Pro Ile Glu Glu Trp Gln His
385                 390                 395                 400

ata gaa cag cta aag ggt agt gct gaa gga agc ata cga tac ccc gca    1248
Ile Glu Gln Leu Lys Gly Ser Ala Glu Gly Ser Ile Arg Tyr Pro Ala
                405                 410                 415
```

```
tgg aat ggg ata ata tca cag gag gta cta gac tac ctt tca tcc tac    1296
Trp Asn Gly Ile Ile Ser Gln Glu Val Leu Asp Tyr Leu Ser Ser Tyr
            420                 425                 430

ata aat aga cgc ata taatga                                         1317
Ile Asn Arg Arg Ile
        435
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<210> 4
<211> 437
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: DNA sequence coding for a fusion protein TAT-Flpe

<400> 4

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Met Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Gly Met Ser
  1               5                  10                  15

Gln Phe Asp Ile Leu Cys Lys Thr Pro Pro Lys Val Leu Val Arg Gln
            20                  25                  30

Phe Val Glu Arg Phe Glu Arg Pro Ser Gly Glu Lys Ile Ala Ser Cys
        35                  40                  45

Ala Ala Glu Leu Thr Tyr Leu Cys Trp Met Ile Thr His Asn Gly Thr
    50                  55                  60

Ala Ile Lys Arg Ala Thr Phe Met Ser Tyr Asn Thr Ile Ile Ser Asn
 65                  70                  75                  80

Ser Leu Ser Phe Asp Ile Val Asn Lys Ser Leu Gln Phe Lys Tyr Lys
                 85                  90                  95

Thr Gln Lys Ala Thr Ile Leu Glu Ala Ser Leu Lys Lys Leu Ile Pro
            100                 105                 110

Ala Trp Glu Phe Thr Ile Ile Pro Tyr Asn Gly Gln Lys His Gln Ser
        115                 120                 125

Asp Ile Thr Asp Ile Val Ser Ser Leu Gln Leu Gln Phe Glu Ser Ser
    130                 135                 140

Glu Glu Ala Asp Lys Gly Asn Ser His Ser Lys Lys Met Leu Lys Ala
145                 150                 155                 160

Leu Leu Ser Glu Gly Glu Ser Ile Trp Glu Ile Thr Glu Lys Ile Leu
                165                 170                 175

Asn Ser Phe Glu Tyr Thr Ser Arg Phe Thr Lys Thr Lys Thr Leu Tyr
            180                 185                 190

Gln Phe Leu Phe Leu Ala Thr Phe Ile Asn Cys Gly Arg Phe Ser Asp
        195                 200                 205

Ile Lys Asn Val Asp Pro Lys Ser Phe Lys Leu Val Gln Asn Lys Tyr
    210                 215                 220

Leu Gly Val Ile Ile Gln Cys Leu Val Thr Glu Thr Lys Thr Ser Val
225                 230                 235                 240
```

Ser Arg His Ile Tyr Phe Phe Ser Ala Arg Gly Arg Ile Asp Pro Leu
245 250 255

Val Tyr Leu Asp Glu Phe Leu Arg Asn Ser Glu Pro Val Leu Lys Arg
260 265 270

Val Asn Arg Thr Gly Asn Ser Ser Ser Asn Lys Gln Glu Tyr Gln Leu
275 280 285

Leu Lys Asp Asn Leu Val Arg Ser Tyr Asn Lys Ala Leu Lys Lys Asn
290 295 300

Ala Pro Tyr Pro Ile Phe Ala Ile Lys Asn Gly Pro Lys Ser His Ile
305 310 315 320

Gly Arg His Leu Met Thr Ser Phe Leu Ser Met Lys Gly Leu Thr Glu
325 330 335

Leu Thr Asn Val Val Gly Asn Trp Ser Asp Lys Arg Ala Ser Ala Val
340 345 350

Ala Arg Thr Thr Tyr Thr His Gln Ile Thr Ala Ile Pro Asp His Tyr
355 360 365

Phe Ala Leu Val Ser Arg Tyr Tyr Ala Tyr Asp Pro Ile Ser Lys Glu
370 375 380

Met Ile Ala Leu Lys Asp Glu Thr Asn Pro Ile Glu Glu Trp Gln His
385 390 395 400

Ile Glu Gln Leu Lys Gly Ser Ala Glu Gly Ser Ile Arg Tyr Pro Ala
405 410 415

Trp Asn Gly Ile Ile Ser Gln Glu Val Leu Asp Tyr Leu Ser Ser Tyr
420 425 430

Ile Asn Arg Arg Ile
435

<210> 5
<211> 2004
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA sequence coding for a fusion protein VP22-Cre

<220>
<221> CDS
<222> (1)..(2001)

<400> 5
atg acc tct cgc cgc tcc gtg aag tcg ggt ccg cgg gag gtt ccg cgc 48
Met Thr Ser Arg Arg Ser Val Lys Ser Gly Pro Arg Glu Val Pro Arg
1 5 10 15

gat gag tac gag gat ctg tac tac acc ccg tct tca ggt atg gcg agt 96
Asp Glu Tyr Glu Asp Leu Tyr Tyr Thr Pro Ser Ser Gly Met Ala Ser
20 25 30

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ccc gat agt ccg cct gac acc tcc cgc cgt ggc gcc cta cag aca cgc    144
Pro Asp Ser Pro Pro Asp Thr Ser Arg Arg Gly Ala Leu Gln Thr Arg
         35                  40                  45

tcg cgc cag agg ggc gag gtc cgt ttc gtc cag tac gac gag tcg gat    192
Ser Arg Gln Arg Gly Glu Val Arg Phe Val Gln Tyr Asp Glu Ser Asp
     50                  55                  60

tat gcc ctc tac ggg ggc tcg tct tcc gaa gac gac gaa cac ccg gag    240
Tyr Ala Leu Tyr Gly Gly Ser Ser Ser Glu Asp Asp Glu His Pro Glu
 65                  70                  75                  80

gtc ccc cgg acg cgg cgt ccc gtt tcc ggg gcg gtt ttg tcc ggc ccg    288
Val Pro Arg Thr Arg Arg Pro Val Ser Gly Ala Val Leu Ser Gly Pro
                 85                  90                  95

ggg cct gcg cgg gcg cct ccg cca ccc gct ggg tcc gga ggg gcc gga    336
Gly Pro Ala Arg Ala Pro Pro Pro Pro Ala Gly Ser Gly Gly Ala Gly
            100                 105                 110

cgc aca ccc acc acc gcc ccc cgg gcc ccc cga acc cag cgg gtg gcg    384
Arg Thr Pro Thr Thr Ala Pro Arg Ala Pro Arg Thr Gln Arg Val Ala
        115                 120                 125

act aag gcc ccc gcg gcc ccg gcg gcg gag acc acc cgc ggc agg aaa    432
Thr Lys Ala Pro Ala Ala Pro Ala Ala Glu Thr Thr Arg Gly Arg Lys
    130                 135                 140

tcg gcc cag cca gaa tcc gcc gca ctc cca gac gcc ccc gcg tcg acg    480
Ser Ala Gln Pro Glu Ser Ala Ala Leu Pro Asp Ala Pro Ala Ser Thr
145                 150                 155                 160

gcg cca acc cga tcc aag aca ccc gcg cag ggg ctg gcc aga aag ctg    528
Ala Pro Thr Arg Ser Lys Thr Pro Ala Gln Gly Leu Ala Arg Lys Leu
                165                 170                 175

cac ttt agc acc gcc ccc cca aac ccc gac gcg cca tgg acc ccc cgg    576
His Phe Ser Thr Ala Pro Pro Asn Pro Asp Ala Pro Trp Thr Pro Arg
            180                 185                 190

gtg gcc ggc ttt aac aag cgc gtc ttc tgc gcc gcg gtc ggg cgc ctg    624
Val Ala Gly Phe Asn Lys Arg Val Phe Cys Ala Ala Val Gly Arg Leu
        195                 200                 205

gcg gcc atg cat gcc cgg atg gcg gcg gtc cag ctc tgg gac atg tcg    672
Ala Ala Met His Ala Arg Met Ala Ala Val Gln Leu Trp Asp Met Ser
    210                 215                 220

cgt ccg cgc aca gac gaa gac ctc aac gaa ctc ctt ggc atc acc acc    720
Arg Pro Arg Thr Asp Glu Asp Leu Asn Glu Leu Leu Gly Ile Thr Thr
225                 230                 235                 240

atc cgc gtg acg gtc tgc gag ggc aaa aac ctg ctt cag cgc gcc aac    768
Ile Arg Val Thr Val Cys Glu Gly Lys Asn Leu Leu Gln Arg Ala Asn
                245                 250                 255

gag ttg gtg aat cca gac gtg gtg cag gac gtc gac gcg gcc acg gcg    816
Glu Leu Val Asn Pro Asp Val Val Gln Asp Val Asp Ala Ala Thr Ala
            260                 265                 270

act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc    864
Thr Arg Gly Arg Ser Ala Ala Ser Arg Pro Thr Glu Arg Pro Arg Ala
        275                 280                 285
```

```
cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag ggt acc gag    912
Pro Ala Arg Ser Ala Ser Arg Pro Arg Arg Pro Val Glu Gly Thr Glu
    290                 295                 300

ctc gga tcc act agt cca gtg tgg tgg aat tct gca gat atc cag cac    960
Leu Gly Ser Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His
305                 310                 315                 320

agt ggc ggc cgc atg tcc aat tta ctg acc gta cac caa aat ttg cct   1008
Ser Gly Gly Arg Met Ser Asn Leu Leu Thr Val His Gln Asn Leu Pro
                325                 330                 335

gca tta ccg gtc gat gca acg agt gat gag gtt cgc aag aac ctg atg   1056
Ala Leu Pro Val Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met
            340                 345                 350

gac atg ttc agg gat cgc cag gcg ttt tct gag cat acc tgg aaa atg   1104
Asp Met Phe Arg Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met
        355                 360                 365

ctt ctg tcc gtt tgc cgg tcg tgg gcg gca tgg tgc aag ttg aat aac   1152
Leu Leu Ser Val Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn
    370                 375                 380

cgg aaa tgg ttt ccc gca gaa cct gaa gat gtt cgc gat tat ctt cta   1200
Arg Lys Trp Phe Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu
385                 390                 395                 400

tat ctt cag gcg cgc ggt ctg gca gta aaa act atc cag caa cat ttg   1248
Tyr Leu Gln Ala Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu
                405                 410                 415

ggc cag cta aac atg ctt cat cgt cgg tcc ggg ctg cca cga cca agt   1296
Gly Gln Leu Asn Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser
            420                 425                 430

gac agc aat gct gtt tca ctg gtt atg cgg cgg atc cga aaa gaa aac   1344
Asp Ser Asn Ala Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn
        435                 440                 445

gtt gat gcc ggt gaa cgt gca aaa cag gct cta gcg ttc gaa cgc act   1392
Val Asp Ala Gly Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr
    450                 455                 460

gat ttc gac cag gtt cgt tca ctc atg gaa aat agc gat cgc tgc cag   1440
Asp Phe Asp Gln Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln
465                 470                 475                 480

gat ata cgt aat ctg gca ttt ctg ggg att gct tat aac acc ctg tta   1488
Asp Ile Arg Asn Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu
                485                 490                 495

cgt ata gcc gaa att gcc agg atc agg gtt aaa gat atc tca cgt act   1536
Arg Ile Ala Glu Ile Ala Arg Ile Arg Val Lys Asp Ile Ser Arg Thr
            500                 505                 510

gac ggt ggg aga atg tta atc cat att ggc aga acg aaa acg ctg gtt   1584
Asp Gly Gly Arg Met Leu Ile His Ile Gly Arg Thr Lys Thr Leu Val
        515                 520                 525

agc acc gca ggt gta gag aag gca ctt agc ctg ggg gta act aaa ctg   1632
Ser Thr Ala Gly Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu
    530                 535                 540
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gtc gag cga tgg att tcc gtc tct ggt gta gct gat gat ccg aat aac 1680
Val Glu Arg Trp Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn
545 550 555 560

tac ctg ttt tgc cgg gtc aga aaa aat ggt gtt gcc gcg cca tct gcc 1728
Tyr Leu Phe Cys Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala
565 570 575

acc agc cag cta tca act cgc gcc ctg gaa ggg att ttt gaa gca act 1776
Thr Ser Gln Leu Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr
580 585 590

cat cga ttg att tac ggc gct aag gat gac tct ggt cag aga tac ctg 1824
His Arg Leu Ile Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu
595 600 605

gcc tgg tct gga cac agt gcc cgt gtc gga gcc gcg cga gat atg gcc 1872
Ala Trp Ser Gly His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala
610 615 620

cgc gct gga gtt tca ata ccg gag atc atg caa gct ggt ggc tgg acc 1920
Arg Ala Gly Val Ser Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr
625 630 635 640

aat gta aat att gtc atg aac tat atc cgt aac ctg gat agt gaa aca 1968
Asn Val Asn Ile Val Met Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr
645 650 655

ggg gca atg gtg cgc ctg ctg gaa gat ggc gat tag 2004
Gly Ala Met Val Arg Leu Leu Glu Asp Gly Asp
660 665

<210> 6
<211> 667
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: DNA sequence
coding for a fusion protein VP22-Cre

<400> 6
Met Thr Ser Arg Arg Ser Val Lys Ser Gly Pro Arg Glu Val Pro Arg
1 5 10 15

Asp Glu Tyr Glu Asp Leu Tyr Tyr Thr Pro Ser Ser Gly Met Ala Ser
20 25 30

Pro Asp Ser Pro Pro Asp Thr Ser Arg Arg Gly Ala Leu Gln Thr Arg
35 40 45

Ser Arg Gln Arg Gly Glu Val Arg Phe Val Gln Tyr Asp Glu Ser Asp
50 55 60

Tyr Ala Leu Tyr Gly Gly Ser Ser Ser Glu Asp Asp Glu His Pro Glu
65 70 75 80

Val Pro Arg Thr Arg Arg Pro Val Ser Gly Ala Val Leu Ser Gly Pro
85 90 95

Gly Pro Ala Arg Ala Pro Pro Pro Pro Ala Gly Ser Gly Gly Ala Gly
100 105 110

Arg Thr Pro Thr Thr Ala Pro Arg Ala Pro Arg Thr Gln Arg Val Ala
115 120 125

Thr Lys Ala Pro Ala Ala Pro Ala Ala Glu Thr Thr Arg Gly Arg Lys
130 135 140

Ser Ala Gln Pro Glu Ser Ala Ala Leu Pro Asp Ala Pro Ala Ser Thr
145 150 155 160

Ala Pro Thr Arg Ser Lys Thr Pro Ala Gln Gly Leu Ala Arg Lys Leu
165 170 175

His Phe Ser Thr Ala Pro Pro Asn Pro Asp Ala Pro Trp Thr Pro Arg
180 185 190

Val Ala Gly Phe Asn Lys Arg Val Phe Cys Ala Ala Val Gly Arg Leu
195 200 205

Ala Ala Met His Ala Arg Met Ala Ala Val Gln Leu Trp Asp Met Ser
210 215 220

Arg Pro Arg Thr Asp Glu Asp Leu Asn Glu Leu Leu Gly Ile Thr Thr
225 230 235 240

Ile Arg Val Thr Val Cys Glu Gly Lys Asn Leu Leu Gln Arg Ala Asn
245 250 255

Glu Leu Val Asn Pro Asp Val Val Gln Asp Val Asp Ala Ala Thr Ala
260 265 270

Thr Arg Gly Arg Ser Ala Ala Ser Arg Pro Thr Glu Arg Pro Arg Ala
275 280 285

Pro Ala Arg Ser Ala Ser Arg Pro Arg Arg Pro Val Glu Gly Thr Glu
290 295 300

Leu Gly Ser Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His
305 310 315 320

Ser Gly Gly Arg Met Ser Asn Leu Leu Thr Val His Gln Asn Leu Pro
325 330 335

Ala Leu Pro Val Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met
340 345 350

Asp Met Phe Arg Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met
355 360 365

Leu Leu Ser Val Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn
370 375 380

Arg Lys Trp Phe Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu
385 390 395 400

Tyr Leu Gln Ala Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu
405 410 415

Gly Gln Leu Asn Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser
420 425 430

Asp Ser Asn Ala Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn
435 440 445

Val Asp Ala Gly Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr
450 455 460

Asp Phe Asp Gln Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln
465 470 475 480

Asp Ile Arg Asn Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu
485 490 495

Arg Ile Ala Glu Ile Ala Arg Ile Arg Val Lys Asp Ile Ser Arg Thr
500 505 510

Asp Gly Gly Arg Met Leu Ile His Ile Gly Arg Thr Lys Thr Leu Val
515 520 525

Ser Thr Ala Gly Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu
530 535 540

Val Glu Arg Trp Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn
545 550 555 560

Tyr Leu Phe Cys Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala
565 570 575

Thr Ser Gln Leu Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr
580 585 590

His Arg Leu Ile Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu
595 600 605

Ala Trp Ser Gly His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala
610 615 620

Arg Ala Gly Val Ser Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr
625 630 635 640

Asn Val Asn Ile Val Met Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr
645 650 655

Gly Ala Met Val Arg Leu Leu Glu Asp Gly Asp
660 665

<210> 7
<211> 2247
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA sequence coding for a fusion protein VP22-Flpe

<220>
<221> CDS
<222> (1)..(2241)

<400> 7
atg acc tct cgc cgc tcc gtg aag tcg ggt ccg cgg gag gtt ccg cgc 48
Met Thr Ser Arg Arg Ser Val Lys Ser Gly Pro Arg Glu Val Pro Arg
1 5 10 15

gat gag tac gag gat ctg tac tac acc ccg tct tca ggt atg gcg agt 96
Asp Glu Tyr Glu Asp Leu Tyr Tyr Thr Pro Ser Ser Gly Met Ala Ser
20 25 30

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ccc gat agt ccg cct gac acc tcc cgc cgt ggc gcc cta cag aca cgc      144
Pro Asp Ser Pro Pro Asp Thr Ser Arg Arg Gly Ala Leu Gln Thr Arg
         35                  40                  45

tcg cgc cag agg ggc gag gtc cgt ttc gtc cag tac gac gag tcg gat      192
Ser Arg Gln Arg Gly Glu Val Arg Phe Val Gln Tyr Asp Glu Ser Asp
     50                  55                  60

tat gcc ctc tac ggg ggc tcg tct tcc gaa gac gac gaa cac ccg gag      240
Tyr Ala Leu Tyr Gly Gly Ser Ser Ser Glu Asp Asp Glu His Pro Glu
 65                  70                  75                  80

gtc ccc cgg acg cgg cgt ccc gtt tcc ggg gcg gtt ttg tcc ggc ccg      288
Val Pro Arg Thr Arg Arg Pro Val Ser Gly Ala Val Leu Ser Gly Pro
                 85                  90                  95

ggg cct gcg cgg gcg cct ccg cca ccc gct ggg tcc gga ggg gcc gga      336
Gly Pro Ala Arg Ala Pro Pro Pro Pro Ala Gly Ser Gly Gly Ala Gly
            100                 105                 110

cgc aca ccc acc acc gcc ccc cgg gcc ccc cga acc cag cgg gtg gcg      384
Arg Thr Pro Thr Thr Ala Pro Arg Ala Pro Arg Thr Gln Arg Val Ala
        115                 120                 125

act aag gcc ccc gcg gcc ccg gcg gcg gag acc acc cgc ggc agg aaa      432
Thr Lys Ala Pro Ala Ala Pro Ala Ala Glu Thr Thr Arg Gly Arg Lys
    130                 135                 140

tcg gcc cag cca gaa tcc gcc gca ctc cca gac gcc ccc gcg tcg acg      480
Ser Ala Gln Pro Glu Ser Ala Ala Leu Pro Asp Ala Pro Ala Ser Thr
145                 150                 155                 160

gcg cca acc cga tcc aag aca ccc gcg cag ggg ctg gcc aga aag ctg      528
Ala Pro Thr Arg Ser Lys Thr Pro Ala Gln Gly Leu Ala Arg Lys Leu
                165                 170                 175

cac ttt agc acc gcc ccc cca aac ccc gac gcg cca tgg acc ccc cgg      576
His Phe Ser Thr Ala Pro Pro Asn Pro Asp Ala Pro Trp Thr Pro Arg
            180                 185                 190

gtg gcc ggc ttt aac aag cgc gtc ttc tgc gcc gcg gtc ggg cgc ctg      624
Val Ala Gly Phe Asn Lys Arg Val Phe Cys Ala Ala Val Gly Arg Leu
        195                 200                 205

gcg gcc atg cat gcc cgg atg gcg gcg gtc cag ctc tgg gac atg tcg      672
Ala Ala Met His Ala Arg Met Ala Ala Val Gln Leu Trp Asp Met Ser
    210                 215                 220

cgt ccg cgc aca gac gaa gac ctc aac gaa ctc ctt ggc atc acc acc      720
Arg Pro Arg Thr Asp Glu Asp Leu Asn Glu Leu Leu Gly Ile Thr Thr
225                 230                 235                 240

atc cgc gtg acg gtc tgc gag ggc aaa aac ctg ctt cag cgc gcc aac      768
Ile Arg Val Thr Val Cys Glu Gly Lys Asn Leu Leu Gln Arg Ala Asn
                245                 250                 255

gag ttg gtg aat cca gac gtg gtg cag gac gtc gac gcg gcc acg gcg      816
Glu Leu Val Asn Pro Asp Val Val Gln Asp Val Asp Ala Ala Thr Ala
            260                 265                 270

act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc      864
Thr Arg Gly Arg Ser Ala Ala Ser Arg Pro Thr Glu Arg Pro Arg Ala
        275                 280                 285
```

cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag ggt acc gag 912
Pro Ala Arg Ser Ala Ser Arg Pro Arg Arg Pro Val Glu Gly Thr Glu
290 295 300

ctc gga tcc act agt cca gtg tgg tgg aat tct gca gat atc cag cac 960
Leu Gly Ser Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His
305 310 315 320

agt ggc ggc cgc atg agt caa ttt gat ata tta tgt aaa aca cca cct 1008
Ser Gly Gly Arg Met Ser Gln Phe Asp Ile Leu Cys Lys Thr Pro Pro
325 330 335

aag gtc ctg gtt cgt cag ttt gtg gaa agg ttt gaa aga cct tca ggg 1056
Lys Val Leu Val Arg Gln Phe Val Glu Arg Phe Glu Arg Pro Ser Gly
340 345 350

gaa aaa ata gca tca tgt gct gct gaa cta acc tat tta tgt tgg atg 1104
Glu Lys Ile Ala Ser Cys Ala Ala Glu Leu Thr Tyr Leu Cys Trp Met
355 360 365

att act cat aac gga aca gca atc aag aga gcc aca ttc atg agc tat 1152
Ile Thr His Asn Gly Thr Ala Ile Lys Arg Ala Thr Phe Met Ser Tyr
370 375 380

aat act atc ata agc aat tcg ctg agt ttc gat att gtc aac aaa tca 1200
Asn Thr Ile Ile Ser Asn Ser Leu Ser Phe Asp Ile Val Asn Lys Ser
385 390 395 400

ctc cag ttt aaa tac aag acg caa aaa gca aca att ctg gaa gcc tca 1248
Leu Gln Phe Lys Tyr Lys Thr Gln Lys Ala Thr Ile Leu Glu Ala Ser
405 410 415

tta aag aaa tta att cct gct tgg gaa ttt aca att att cct tac aat 1296
Leu Lys Lys Leu Ile Pro Ala Trp Glu Phe Thr Ile Ile Pro Tyr Asn
420 425 430

gga caa aaa cat caa tct gat atc act gat att gta agt agt ttg caa 1344
Gly Gln Lys His Gln Ser Asp Ile Thr Asp Ile Val Ser Ser Leu Gln
435 440 445

tta cag ttc gaa tca tcg gaa gaa gca gat aag gga aat agc cac agt 1392
Leu Gln Phe Glu Ser Ser Glu Glu Ala Asp Lys Gly Asn Ser His Ser
450 455 460

aaa aaa atg ctt aaa gca ctt cta agt gag ggt gaa agc atc tgg gag 1440
Lys Lys Met Leu Lys Ala Leu Leu Ser Glu Gly Glu Ser Ile Trp Glu
465 470 475 480

atc act gag aaa ata cta aat tcg ttt gag tat acc tcg aga ttt aca 1488
Ile Thr Glu Lys Ile Leu Asn Ser Phe Glu Tyr Thr Ser Arg Phe Thr
485 490 495

aaa aca aaa act tta tac caa ttc ctc ttc cta gct act ttc atc aat 1536
Lys Thr Lys Thr Leu Tyr Gln Phe Leu Phe Leu Ala Thr Phe Ile Asn
500 505 510

tgt gga aga ttc agc gat att aag aac gtt gat ccg aaa tca ttt aaa 1584
Cys Gly Arg Phe Ser Asp Ile Lys Asn Val Asp Pro Lys Ser Phe Lys
515 520 525

tta gtc caa aat aag tat ctg gga gta ata atc cag tgt tta gtg aca 1632
Leu Val Gln Asn Lys Tyr Leu Gly Val Ile Ile Gln Cys Leu Val Thr
530 535 540

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gag aca aag aca agc gtt agt agg cac ata tac ttc ttt agc gca agg    1680
Glu Thr Lys Thr Ser Val Ser Arg His Ile Tyr Phe Phe Ser Ala Arg
545                 550                 555                 560

ggt agg atc gat cca ctt gta tat ttg gat gaa ttt ttg agg aat tct    1728
Gly Arg Ile Asp Pro Leu Val Tyr Leu Asp Glu Phe Leu Arg Asn Ser
                565                 570                 575

gaa cca gtc cta aaa cga gta aat agg acc ggc aat tct tca agc aac    1776
Glu Pro Val Leu Lys Arg Val Asn Arg Thr Gly Asn Ser Ser Ser Asn
            580                 585                 590

aaa cag gaa tac caa tta tta aaa gat aac tta gtc aga tcg tac aac    1824
Lys Gln Glu Tyr Gln Leu Leu Lys Asp Asn Leu Val Arg Ser Tyr Asn
        595                 600                 605

aag gct ttg aag aaa aat gcg cct tat cca atc ttt gct ata aag aat    1872
Lys Ala Leu Lys Lys Asn Ala Pro Tyr Pro Ile Phe Ala Ile Lys Asn
    610                 615                 620

ggc cca aaa tct cac att gga aga cat ttg atg acc tca ttt ctg tca    1920
Gly Pro Lys Ser His Ile Gly Arg His Leu Met Thr Ser Phe Leu Ser
625                 630                 635                 640

atg aag ggc cta acg gag ttg act aat gtt gtg gga aat tgg agc gat    1968
Met Lys Gly Leu Thr Glu Leu Thr Asn Val Val Gly Asn Trp Ser Asp
                645                 650                 655

aag cgt gct tct gcc gtg gcc agg aca acg tat act cat cag ata aca    2016
Lys Arg Ala Ser Ala Val Ala Arg Thr Thr Tyr Thr His Gln Ile Thr
            660                 665                 670

gca ata cct gat cac tac ttc gca cta gtt tct cgg tac tat gca tat    2064
Ala Ile Pro Asp His Tyr Phe Ala Leu Val Ser Arg Tyr Tyr Ala Tyr
        675                 680                 685

gat cca ata tca aag gaa atg ata gca ttg aag gat gag act aat cca    2112
Asp Pro Ile Ser Lys Glu Met Ile Ala Leu Lys Asp Glu Thr Asn Pro
    690                 695                 700

att gag gag tgg cag cat ata gaa cag cta aag ggt agt gct gaa gga    2160
Ile Glu Glu Trp Gln His Ile Glu Gln Leu Lys Gly Ser Ala Glu Gly
705                 710                 715                 720

agc ata cga tac ccc gca tgg aat ggg ata ata tca cag gag gta cta    2208
Ser Ile Arg Tyr Pro Ala Trp Asn Gly Ile Ile Ser Gln Glu Val Leu
                725                 730                 735

gac tac ctt tca tcc tac ata aat aga cgc ata taatga                 2247
Asp Tyr Leu Ser Ser Tyr Ile Asn Arg Arg Ile
            740                 745
```

<210> 8
<211> 747
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: DNA sequence coding for a fusion protein VP22-Flpe

<400> 8

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Met Thr Ser Arg Arg Ser Val Lys Ser Gly Pro Arg Glu Val Pro Arg
  1               5                  10                  15
```

```
Asp Glu Tyr Glu Asp Leu Tyr Tyr Thr Pro Ser Ser Gly Met Ala Ser
            20                  25                  30

Pro Asp Ser Pro Pro Asp Thr Ser Arg Arg Gly Ala Leu Gln Thr Arg
        35                  40                  45

Ser Arg Gln Arg Gly Glu Val Arg Phe Val Gln Tyr Asp Glu Ser Asp
    50                  55                  60

Tyr Ala Leu Tyr Gly Gly Ser Ser Ser Glu Asp Asp Glu His Pro Glu
65                  70                  75                  80

Val Pro Arg Thr Arg Arg Pro Val Ser Gly Ala Val Leu Ser Gly Pro
                85                  90                  95

Gly Pro Ala Arg Ala Pro Pro Pro Pro Ala Gly Ser Gly Gly Ala Gly
            100                 105                 110

Arg Thr Pro Thr Thr Ala Pro Arg Ala Pro Arg Thr Gln Arg Val Ala
        115                 120                 125

Thr Lys Ala Pro Ala Ala Pro Ala Ala Glu Thr Thr Arg Gly Arg Lys
    130                 135                 140

Ser Ala Gln Pro Glu Ser Ala Ala Leu Pro Asp Ala Pro Ala Ser Thr
145                 150                 155                 160

Ala Pro Thr Arg Ser Lys Thr Pro Ala Gln Gly Leu Ala Arg Lys Leu
                165                 170                 175

His Phe Ser Thr Ala Pro Pro Asn Pro Asp Ala Pro Trp Thr Pro Arg
            180                 185                 190

Val Ala Gly Phe Asn Lys Arg Val Phe Cys Ala Ala Val Gly Arg Leu
        195                 200                 205

Ala Ala Met His Ala Arg Met Ala Ala Val Gln Leu Trp Asp Met Ser
    210                 215                 220

Arg Pro Arg Thr Asp Glu Asp Leu Asn Glu Leu Leu Gly Ile Thr Thr
225                 230                 235                 240

Ile Arg Val Thr Val Cys Glu Gly Lys Asn Leu Leu Gln Arg Ala Asn
                245                 250                 255

Glu Leu Val Asn Pro Asp Val Val Gln Asp Val Asp Ala Ala Thr Ala
            260                 265                 270

Thr Arg Gly Arg Ser Ala Ala Ser Arg Pro Thr Glu Arg Pro Arg Ala
        275                 280                 285

Pro Ala Arg Ser Ala Ser Arg Pro Arg Arg Pro Val Glu Gly Thr Glu
    290                 295                 300

Leu Gly Ser Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His
305                 310                 315                 320

Ser Gly Gly Arg Met Ser Gln Phe Asp Ile Leu Cys Lys Thr Pro Pro
                325                 330                 335

Lys Val Leu Val Arg Gln Phe Val Glu Arg Phe Glu Arg Pro Ser Gly
            340                 345                 350
```

```
Glu Lys Ile Ala Ser Cys Ala Ala Glu Leu Thr Tyr Leu Cys Trp Met
        355                 360                 365

Ile Thr His Asn Gly Thr Ala Ile Lys Arg Ala Thr Phe Met Ser Tyr
    370                 375                 380

Asn Thr Ile Ile Ser Asn Ser Leu Ser Phe Asp Ile Val Asn Lys Ser
385                 390                 395                 400

Leu Gln Phe Lys Tyr Lys Thr Gln Lys Ala Thr Ile Leu Glu Ala Ser
                405                 410                 415

Leu Lys Lys Leu Ile Pro Ala Trp Glu Phe Thr Ile Ile Pro Tyr Asn
            420                 425                 430

Gly Gln Lys His Gln Ser Asp Ile Thr Asp Ile Val Ser Ser Leu Gln
        435                 440                 445

Leu Gln Phe Glu Ser Ser Glu Glu Ala Asp Lys Gly Asn Ser His Ser
    450                 455                 460

Lys Lys Met Leu Lys Ala Leu Leu Ser Glu Gly Glu Ser Ile Trp Glu
465                 470                 475                 480

Ile Thr Glu Lys Ile Leu Asn Ser Phe Glu Tyr Thr Ser Arg Phe Thr
                485                 490                 495

Lys Thr Lys Thr Leu Tyr Gln Phe Leu Phe Leu Ala Thr Phe Ile Asn
            500                 505                 510

Cys Gly Arg Phe Ser Asp Ile Lys Asn Val Asp Pro Lys Ser Phe Lys
        515                 520                 525

Leu Val Gln Asn Lys Tyr Leu Gly Val Ile Ile Gln Cys Leu Val Thr
    530                 535                 540

Glu Thr Lys Thr Ser Val Ser Arg His Ile Tyr Phe Phe Ser Ala Arg
545                 550                 555                 560

Gly Arg Ile Asp Pro Leu Val Tyr Leu Asp Glu Phe Leu Arg Asn Ser
                565                 570                 575

Glu Pro Val Leu Lys Arg Val Asn Arg Thr Gly Asn Ser Ser Ser Asn
            580                 585                 590

Lys Gln Glu Tyr Gln Leu Leu Lys Asp Asn Leu Val Arg Ser Tyr Asn
        595                 600                 605

Lys Ala Leu Lys Lys Asn Ala Pro Tyr Pro Ile Phe Ala Ile Lys Asn
    610                 615                 620

Gly Pro Lys Ser His Ile Gly Arg His Leu Met Thr Ser Phe Leu Ser
625                 630                 635                 640

Met Lys Gly Leu Thr Glu Leu Thr Asn Val Val Gly Asn Trp Ser Asp
                645                 650                 655

Lys Arg Ala Ser Ala Val Ala Arg Thr Thr Tyr Thr His Gln Ile Thr
            660                 665                 670

Ala Ile Pro Asp His Tyr Phe Ala Leu Val Ser Arg Tyr Tyr Ala Tyr
        675                 680                 685
```

```
Asp Pro Ile Ser Lys Glu Met Ile Ala Leu Lys Asp Glu Thr Asn Pro
    690                 695                 700

Ile Glu Glu Trp Gln His Ile Glu Gln Leu Lys Gly Ser Ala Glu Gly
705                 710                 715                 720

Ser Ile Arg Tyr Pro Ala Trp Asn Gly Ile Ile Ser Gln Glu Val Leu
                725                 730                 735

Asp Tyr Leu Ser Ser Tyr Ile Asn Arg Arg Ile
            740                 745


<210> 9
<211> 33
<212> DNA
<213> Human immunodeficiency virus

<400> 9
tacggccgca agaagcgccg ccaacgccgc cgc                                33


<210> 10
<211> 11
<212> PRT
<213> Human immunodeficiency virus

<400> 10
Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg
  1               5                  10


<210> 11
<211> 42
<212> DNA
<213> Human immunodeficiency virus

<220>
<221> CDS
<222> (4)..(42)

<400> 11
atg ggc tac ggc cgc aag aag cgc cgc caa cgc cgc cgc ggc             42
    Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Gly
      1               5                  10


<210> 12
<211> 13
<212> PRT
<213> Human immunodeficiency virus

<400> 12
Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Gly
  1               5                  10


<210> 13
<211> 1623
<212> DNA
<213> Artificial Sequence

<220>
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<223> Description of Artificial Sequence: DNA sequence
      coding for a fusion protein deltaVP22cre-StrepTag

<220>
<221> CDS
<222> (1)..(1617)

<400> 13
atg gct agc atg act ggt gga cag caa atg ggt cgg gat ccg tcg acg      48
Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Asp Pro Ser Thr
  1               5                  10                  15

gcg cca acc cga tcc aag aca ccc gcg cag ggg ctg gcc aga aag ctg      96
Ala Pro Thr Arg Ser Lys Thr Pro Ala Gln Gly Leu Ala Arg Lys Leu
             20                  25                  30

cac ttt agc acc gcc ccc cca aac ccc gac gcg cca tgg acc ccc cgg     144
His Phe Ser Thr Ala Pro Pro Asn Pro Asp Ala Pro Trp Thr Pro Arg
         35                  40                  45

gtg gcc ggc ttt aac aag cgc gtc ttc tgc gcc gcg gtc ggg cgc ctg     192
Val Ala Gly Phe Asn Lys Arg Val Phe Cys Ala Ala Val Gly Arg Leu
     50                  55                  60

gcg gcc atg cat gcc cgg atg gcg gct gtc cag ctc tgg gac atg tcg     240
Ala Ala Met His Ala Arg Met Ala Ala Val Gln Leu Trp Asp Met Ser
 65                  70                  75                  80

cgt ccg cgc aca gac gaa gac ctc aac gaa ctc ctt ggc atc acc acc     288
Arg Pro Arg Thr Asp Glu Asp Leu Asn Glu Leu Leu Gly Ile Thr Thr
                 85                  90                  95

atc cgc gtg acg gtc tgc gag ggc aaa aac ctg ctt cag cgc gcc aac     336
Ile Arg Val Thr Val Cys Glu Gly Lys Asn Leu Leu Gln Arg Ala Asn
            100                 105                 110

gag ttg gtg aat cca gac gtg gtg cag gac gtc gac gcg gcc acg gcg     384
Glu Leu Val Asn Pro Asp Val Val Gln Asp Val Asp Ala Ala Thr Ala
        115                 120                 125

act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc     432
Thr Arg Gly Arg Ser Ala Ala Ser Arg Pro Thr Glu Arg Pro Arg Ala
    130                 135                 140

cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag ggt acc gag     480
Pro Ala Arg Ser Ala Ser Arg Pro Arg Arg Pro Val Glu Gly Thr Glu
145                 150                 155                 160

ctc gga tcc act agt cca gtg tgg tgg aat tct gca gat atc cag cac     528
Leu Gly Ser Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His
                165                 170                 175

agt ggc ggc cgc atg tcc aat tta ctg acc gta cac caa aat ttg cct     576
Ser Gly Gly Arg Met Ser Asn Leu Leu Thr Val His Gln Asn Leu Pro
            180                 185                 190

gca tta ccg gtc gat gca acg agt gat gag gtt cgc aag aac ctg atg     624
Ala Leu Pro Val Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met
        195                 200                 205

gac atg ttc agg gat cgc cag gcg ttt tct gag cat acc tgg aaa atg     672
Asp Met Phe Arg Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met
    210                 215                 220
```

```
ctt ctg tcc gtt tgc cgg tcg tgg gcg gca tgg tgc aag ttg aat aac    720
Leu Leu Ser Val Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn
225                 230                 235                 240

cgg aaa tgg ttt ccc gca gaa cct gaa gat gtt cgc gat tat ctt cta    768
Arg Lys Trp Phe Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu
                245                 250                 255

tat ctt cag gcg cgc ggt ctg gca gta aaa act atc cag caa cat ttg    816
Tyr Leu Gln Ala Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu
            260                 265                 270

ggc cag cta aac atg ctt cat cgt cgg tcc ggg ctg cca cga cca agt    864
Gly Gln Leu Asn Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser
        275                 280                 285

gac agc aat gct gtt tca ctg gtt atg cgg cgg atc cga aaa gaa aac    912
Asp Ser Asn Ala Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn
    290                 295                 300

gtt gat gcc ggt gaa cgt gca aaa cag gct cta gcg ttc gaa cgc act    960
Val Asp Ala Gly Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr
305                 310                 315                 320

gat ttc gac cag gtt cgt tca ctc atg gaa aat agc gat cgc tgc cag    1008
Asp Phe Asp Gln Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln
                325                 330                 335

gat ata cgt aat ctg gca ttt ctg ggg att gct tat aac acc ctg tta    1056
Asp Ile Arg Asn Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu
            340                 345                 350

cgt ata gcc gaa att gcc agg atc agg gtt aaa gat atc tca cgt act    1104
Arg Ile Ala Glu Ile Ala Arg Ile Arg Val Lys Asp Ile Ser Arg Thr
        355                 360                 365

gac ggt ggg aga atg tta atc cat att ggc aga acg aaa acg ctg gtt    1152
Asp Gly Gly Arg Met Leu Ile His Ile Gly Arg Thr Lys Thr Leu Val
    370                 375                 380

agc acc gca ggt gta gag aag gca ctt agc ctg ggg gta act aaa ctg    1200
Ser Thr Ala Gly Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu
385                 390                 395                 400

gtc gag cga tgg att tcc gtc tct ggt gta gct gat gat ccg aat aac    1248
Val Glu Arg Trp Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn
                405                 410                 415

tac ctg ttt tgc cgg gtc aga aaa aat ggt gtt gcc gcg cca tct gcc    1296
Tyr Leu Phe Cys Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala
            420                 425                 430

acc agc cag cta tca act cgc gcc ctg gaa ggg att ttt gaa gca act    1344
Thr Ser Gln Leu Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr
        435                 440                 445

cat cga ttg att tac ggc gct aag gat gac tct ggt cag aga tac ctg    1392
His Arg Leu Ile Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu
    450                 455                 460

gcc tgg tct gga cac agt gcc cgt gtc gga gcc gcg cga gat atg gcc    1440
Ala Trp Ser Gly His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala
465                 470                 475                 480
```

```
cgc gct gga gtt tca ata ccg gag atc atg caa gct ggt ggc tgg acc    1488
Arg Ala Gly Val Ser Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr
                485                 490                 495

aat gta aat att gtc atg aac tat atc cgt aac ctg gat agt gaa aca    1536
Asn Val Asn Ile Val Met Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr
            500                 505                 510

ggg gca atg gtg cgc ctg ctg gaa gat ggc gat ggt atc gaa ggt cgt    1584
Gly Ala Met Val Arg Leu Leu Glu Asp Gly Asp Gly Ile Glu Gly Arg
        515                 520                 525

ggt agc gct tgg cgt cac ccg cag ttc ggt ggt taataa                 1623
Gly Ser Ala Trp Arg His Pro Gln Phe Gly Gly
    530                 535


<210> 14
<211> 539
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: DNA sequence
      coding for a fusion protein deltaVP22cre-StrepTag

<400> 14
Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Asp Pro Ser Thr
  1               5                  10                  15

Ala Pro Thr Arg Ser Lys Thr Pro Ala Gln Gly Leu Ala Arg Lys Leu
             20                  25                  30

His Phe Ser Thr Ala Pro Pro Asn Pro Asp Ala Pro Trp Thr Pro Arg
         35                  40                  45

Val Ala Gly Phe Asn Lys Arg Val Phe Cys Ala Ala Val Gly Arg Leu
     50                  55                  60

Ala Ala Met His Ala Arg Met Ala Ala Val Gln Leu Trp Asp Met Ser
 65                  70                  75                  80

Arg Pro Arg Thr Asp Glu Asp Leu Asn Glu Leu Leu Gly Ile Thr Thr
                 85                  90                  95

Ile Arg Val Thr Val Cys Glu Gly Lys Asn Leu Leu Gln Arg Ala Asn
            100                 105                 110

Glu Leu Val Asn Pro Asp Val Val Gln Asp Val Asp Ala Ala Thr Ala
        115                 120                 125

Thr Arg Gly Arg Ser Ala Ala Ser Arg Pro Thr Glu Arg Pro Arg Ala
    130                 135                 140

Pro Ala Arg Ser Ala Ser Arg Pro Arg Arg Pro Val Glu Gly Thr Glu
145                 150                 155                 160

Leu Gly Ser Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His
                165                 170                 175

Ser Gly Gly Arg Met Ser Asn Leu Leu Thr Val His Gln Asn Leu Pro
            180                 185                 190

Ala Leu Pro Val Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met
        195                 200                 205
```

```
Asp Met Phe Arg Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met
    210                 215                 220

Leu Leu Ser Val Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn
225                 230                 235                 240

Arg Lys Trp Phe Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu
                245                 250                 255

Tyr Leu Gln Ala Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu
            260                 265                 270

Gly Gln Leu Asn Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser
        275                 280                 285

Asp Ser Asn Ala Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn
    290                 295                 300

Val Asp Ala Gly Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr
305                 310                 315                 320

Asp Phe Asp Gln Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln
                325                 330                 335

Asp Ile Arg Asn Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu
            340                 345                 350

Arg Ile Ala Glu Ile Ala Arg Ile Arg Val Lys Asp Ile Ser Arg Thr
        355                 360                 365

Asp Gly Gly Arg Met Leu Ile His Ile Gly Arg Thr Lys Thr Leu Val
    370                 375                 380

Ser Thr Ala Gly Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu
385                 390                 395                 400

Val Glu Arg Trp Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn
                405                 410                 415

Tyr Leu Phe Cys Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala
            420                 425                 430

Thr Ser Gln Leu Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr
        435                 440                 445

His Arg Leu Ile Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu
    450                 455                 460

Ala Trp Ser Gly His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala
465                 470                 475                 480

Arg Ala Gly Val Ser Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr
                485                 490                 495

Asn Val Asn Ile Val Met Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr
            500                 505                 510

Gly Ala Met Val Arg Leu Leu Glu Asp Gly Asp Gly Ile Glu Gly Arg
        515                 520                 525

Gly Ser Ala Trp Arg His Pro Gln Phe Gly Gly
    530                 535
```

<210> 15
<211> 5953
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: vector
pCRT7-deltaVPCS

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gggatttttg aagcaactca tcgattgatt tacggcgcta aggatgactc tggtcagaga 180
tacctggcct ggtctggaca cagtgcccgt gtcggagccg cgcgagatat ggcccgcgct 240
ggagtttcaa taccggagat catgcaagct ggtggctgga ccaatgtaaa tattgtcatg 300
aactatatcc gtaacctgga tagtgaaaca ggggcaatgg tgcgcctgct ggaagatggc 360
gatggtatcg aaggtcgtgg tagcgcttgg cgtcacccgc agttcggtgg ttaataagct 420
tcgaacaaaa actcatctca gaagaggatc tgaatatgca taccggtcat catcaccatc 480
accattgagt tttgagcaat aactagcata accccttggg gcctctaaac gggtcttgag 540
gggttttttg ctgaaaggag gaactatatc cggatatcca caggacgggt gtggtcgcca 600
tgatcgcgta gtcgatagtg gctccaagta gcgaagcgag caggactggg cggcggccaa 660
agcggtcgga cagtgctccg agaacgggtg cgcatagaaa ttgcatcaac gcatatagcg 720
ctagcagcac gccatagtga ctggcgatgc tgtcggaatg gacgatatcc cgcaagaggc 780
ccggcagtac cggcataacc aagcctatgc ctacagcatc cagggtgacg gtgccgagga 840
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gtgataaact accgcattaa agcttatcga tgataagctg tcaaacatga gaattaattc 960
ttagaaaaac tcatcgagca tcaaatgaaa ctgcaattta ttcatatcag gattatcaat 1020
accatatttt tgaaaaagcc gtttctgtaa tgaaggagaa aactcaccga ggcagttcca 1080
taggatggca agatcctggt atcggtctgc gattccgact cgtccaacat caatacaacc 1140
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tgaatccggt gagaatggca aaagcttatg catttctttc cagacttgtt caacaggcca 1260
gccattacgc tcgtcatcaa aatcactcgc atcaaccaaa ccgttattca ttcgtgattg 1320
cgcctgagcg agacgaaata cgcgatcgct gttaaaagga caattacaaa caggaatcga 1380
atgcaaccgg cgcaggaaca ctgccagcgc atcaacaata ttttcacctg aatcaggata 1440
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tagtctgacc atctcatctg taacatcatt ggcaacgcta cctttgccat gtttcagaaa 1620
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gtaagcagac agttttattg ttcatgacca aaatccctta acgtgagttt tcgttccact 1860
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gcggtatgga tgcggcggga ccagagaaaa atcactcagg gtcaatgcca gcgcttcgtt 3300
aatacagatg taggtgttcc acagggtagc cagcagcatc ctgcgatgca gatccggaac 3360
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Asn Leu Leu Thr Val His Gln Asn Leu Pro Ala Leu Pro Val Asp Ala
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acg agt gat gag gtt cgc aag aac ctg atg gac atg ttc agg gat cgc 144
Thr Ser Asp Glu Val Arg Lys Asn Leu Met Asp Met Phe Arg Asp Arg
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Gln Ala Phe Ser Glu His Thr Trp Lys Met Leu Leu Ser Val Cys Arg
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gaa cct gaa gat gtt cgc gat tat ctt cta tat ctt cag gcg cgc ggt    288
Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu Tyr Leu Gln Ala Arg Gly
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His Arg Arg Ser Gly Leu Pro Arg Pro Ser Asp Ser Asn Ala Val Ser
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Leu Val Met Arg Arg Ile Arg Lys Glu Asn Val Asp Ala Gly Glu Arg
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Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr Asp Phe Asp Gln Val Arg
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Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu Arg Ile Ala Glu Ile Ala
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Arg Ile Arg Val Lys Asp Ile Ser Arg Thr Asp Gly Gly Arg Met Leu
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aag gca ctt agc ctg ggg gta act aaa ctg gtc gag cga tgg att tcc    720
Lys Ala Leu Ser Leu Gly Val Thr Lys Leu Val Glu Arg Trp Ile Ser
225                 230                 235                 240

gtc tct ggt gta gct gat gat ccg aat aac tac ctg ttt tgc cgg gtc    768
Val Ser Gly Val Ala Asp Asp Pro Asn Asn Tyr Leu Phe Cys Arg Val
                245                 250                 255

aga aaa aat ggt gtt gcc gcg cca tct gcc acc agc cag cta tca act    816
Arg Lys Asn Gly Val Ala Ala Pro Ser Ala Thr Ser Gln Leu Ser Thr
            260                 265                 270

cgc gcc ctg gaa ggg att ttt gaa gca act cat cga ttg att tac ggc    864
Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr His Arg Leu Ile Tyr Gly
        275                 280                 285

gct aag gat gac tct ggt cag aga tac ctg gcc tgg tct gga cac agt    912
Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu Ala Trp Ser Gly His Ser
    290                 295                 300

gcc cgt gtc gga gcc gcg cga gat atg gcc cgc gct gga gtt tca ata    960
Ala Arg Val Gly Ala Ala Arg Asp Met Ala Arg Ala Gly Val Ser Ile
305                 310                 315                 320
```

ccg gag atc atg caa gct ggt ggc tgg acc aat gta aat att gtc atg 1008
Pro Glu Ile Met Gln Ala Gly Gly Trp Thr Asn Val Asn Ile Val Met
325 330 335

aac tat atc cgt aac ctg gat agt gaa aca ggg gca atg gtg cgc ctg 1056
Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr Gly Ala Met Val Arg Leu
340 345 350

ctg gaa gat ggc gat ggt atc gaa ggt cgt ggt agc gct tgg cgt cac 1104
Leu Glu Asp Gly Asp Gly Ile Glu Gly Arg Gly Ser Ala Trp Arg His
355 360 365

ccg cag ttc ggt ggt taataa 1125
Pro Gln Phe Gly Gly
370

<210> 19
<211> 373
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: DNA sequence coding for a fusion protein TATcreStrepTag

<400> 19
Met Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Gly Met Ser
1 5 10 15

Asn Leu Leu Thr Val His Gln Asn Leu Pro Ala Leu Pro Val Asp Ala
20 25 30

Thr Ser Asp Glu Val Arg Lys Asn Leu Met Asp Met Phe Arg Asp Arg
35 40 45

Gln Ala Phe Ser Glu His Thr Trp Lys Met Leu Leu Ser Val Cys Arg
50 55 60

Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn Arg Lys Trp Phe Pro Ala
65 70 75 80

Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu Tyr Leu Gln Ala Arg Gly
85 90 95

Leu Thr Val Lys Thr Ile Gln Gln His Leu Gly Gln Leu Asn Met Leu
100 105 110

His Arg Arg Ser Gly Leu Pro Arg Pro Ser Asp Ser Asn Ala Val Ser
115 120 125

Leu Val Met Arg Arg Ile Arg Lys Glu Asn Val Asp Ala Gly Glu Arg
130 135 140

Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr Asp Phe Asp Gln Val Arg
145 150 155 160

Ser Leu Met Glu Asn Ser Asp Arg Cys Gln Asp Ile Arg Asn Leu Ala
165 170 175

Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu Arg Ile Ala Glu Ile Ala
180 185 190

Arg Ile Arg Val Lys Asp Ile Ser Arg Thr Asp Gly Gly Arg Met Leu
195 200 205

Ile His Ile Gly Arg Thr Lys Thr Leu Val Ser Thr Ala Gly Val Glu
    210   215   220

Lys Ala Leu Ser Leu Gly Val Thr Lys Leu Val Glu Arg Trp Ile Ser
225   230   235   240

Val Ser Gly Val Ala Asp Asp Pro Asn Asn Tyr Leu Phe Cys Arg Val
                245   250   255

Arg Lys Asn Gly Val Ala Ala Pro Ser Ala Thr Ser Gln Leu Ser Thr
            260   265   270

Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr His Arg Leu Ile Tyr Gly
        275   280   285

Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu Ala Trp Ser Gly His Ser
    290   295   300

Ala Arg Val Gly Ala Ala Arg Asp Met Ala Arg Ala Gly Val Ser Ile
305   310   315   320

Pro Glu Ile Met Gln Ala Gly Gly Trp Thr Asn Val Asn Ile Val Met
                325   330   335

Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr Gly Ala Met Val Arg Leu
            340   345   350

Leu Glu Asp Gly Asp Gly Ile Glu Gly Arg Gly Ser Ala Trp Arg His
        355   360   365

Pro Gln Phe Gly Gly
    370

<210> 20
<211> 2055
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA sequence
coding for a fusion protein VP22creStrepTag

<220>
<221> CDS
<222> (1)..(2049)

<400> 20
atg acc tct cgc cgc tcc gtg aag tcg ggt ccg cgg gag gtt ccg cgc 48
Met Thr Ser Arg Arg Ser Val Lys Ser Gly Pro Arg Glu Val Pro Arg
1   5   10   15

gat gag tac gag gat ctg tac tac acc ccg tct tca ggt atg gcg agt 96
Asp Glu Tyr Glu Asp Leu Tyr Tyr Thr Pro Ser Ser Gly Met Ala Ser
            20   25   30

ccc gat agt ccg cct gac acc tcc cgc cgt ggc gcc cta cag aca cgc 144
Pro Asp Ser Pro Pro Asp Thr Ser Arg Arg Gly Ala Leu Gln Thr Arg
        35   40   45

tcg cgc cag agg ggc gag gtc cgt ttc gtc cag tac gac gag tcg gat 192
Ser Arg Gln Arg Gly Glu Val Arg Phe Val Gln Tyr Asp Glu Ser Asp
    50   55   60

```
tat gcc ctc tac ggg ggc tcg tct tcc gaa gac gac gaa cac ccg gag   240
Tyr Ala Leu Tyr Gly Gly Ser Ser Ser Glu Asp Asp Glu His Pro Glu
 65                  70                  75                  80

gtc ccc cgg acg cgg cgt ccc gtt tcc ggg gcg gtt ttg tcc ggc ccg   288
Val Pro Arg Thr Arg Arg Pro Val Ser Gly Ala Val Leu Ser Gly Pro
                 85                  90                  95

ggg cct gcg cgg gcg cct ccg cca ccc gct ggg tcc gga ggg gcc gga   336
Gly Pro Ala Arg Ala Pro Pro Pro Pro Ala Gly Ser Gly Gly Ala Gly
            100                 105                 110

cgc aca ccc acc acc gcc ccc cgg gcc ccc cga acc cag cgg gtg gcg   384
Arg Thr Pro Thr Thr Ala Pro Arg Ala Pro Arg Thr Gln Arg Val Ala
        115                 120                 125

tct aag gcc ccc gcg gcc ccg gcg gcg gag acc acc cgc ggc agg aaa   432
Ser Lys Ala Pro Ala Ala Pro Ala Ala Glu Thr Thr Arg Gly Arg Lys
    130                 135                 140

tcg gcc cag cca gaa tcc gcc gca ctc cca gac gcc ccc gcg tcg acg   480
Ser Ala Gln Pro Glu Ser Ala Ala Leu Pro Asp Ala Pro Ala Ser Thr
145                 150                 155                 160

gcg cca acc cga tcc aag aca ccc gcg cag ggg ctg gcc aga aag ctg   528
Ala Pro Thr Arg Ser Lys Thr Pro Ala Gln Gly Leu Ala Arg Lys Leu
                165                 170                 175

cac ttt agc acc gcc ccc cca aac ccc gac gcg cca tgg acc ccc cgg   576
His Phe Ser Thr Ala Pro Pro Asn Pro Asp Ala Pro Trp Thr Pro Arg
            180                 185                 190

gtg gcc ggc ttt aac aag cgc gtc ttc tgc gcc gcg gtc ggg cgc ctg   624
Val Ala Gly Phe Asn Lys Arg Val Phe Cys Ala Ala Val Gly Arg Leu
        195                 200                 205

gcg gcc atg cat gcc cgg atg gcg gct gtc cag ctc tgg gac atg tcg   672
Ala Ala Met His Ala Arg Met Ala Ala Val Gln Leu Trp Asp Met Ser
    210                 215                 220

cgt ccg cgc aca gac gaa gac ctc aac gaa ctc ctt ggc atc acc acc   720
Arg Pro Arg Thr Asp Glu Asp Leu Asn Glu Leu Leu Gly Ile Thr Thr
225                 230                 235                 240

atc cgc gtg acg gtc tgc gag ggc aaa aac ctg ctt cag cgc gcc aac   768
Ile Arg Val Thr Val Cys Glu Gly Lys Asn Leu Leu Gln Arg Ala Asn
                245                 250                 255

gag ttg gtg aat cca gac gtg gtg cag gac gtc gac gcg gcc acg gcg   816
Glu Leu Val Asn Pro Asp Val Val Gln Asp Val Asp Ala Ala Thr Ala
            260                 265                 270

act cga ggg cgt tct gcg gcg tcg cgc ccc acc gag cga cct cga gcc   864
Thr Arg Gly Arg Ser Ala Ala Ser Arg Pro Thr Glu Arg Pro Arg Ala
        275                 280                 285

cca gcc cgc tcc gct tct cgc ccc aga cgg ccc gtc gag ggt acc gag   912
Pro Ala Arg Ser Ala Ser Arg Pro Arg Arg Pro Val Glu Gly Thr Glu
    290                 295                 300

ctc gga tcc act agt cca gtg tgg tgg aat tct gca gat atc cag cac   960
Leu Gly Ser Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His
305                 310                 315                 320
```

```
agt ggc ggc cgc atg tcc aat tta ctg acc gta cac caa aat ttg cct    1008
Ser Gly Gly Arg Met Ser Asn Leu Leu Thr Val His Gln Asn Leu Pro
                325                 330                 335

gca tta ccg gtc gat gca acg agt gat gag gtt cgc aag aac ctg atg    1056
Ala Leu Pro Val Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met
            340                 345                 350

gac atg ttc agg gat cgc cag gcg ttt tct gag cat acc tgg aaa atg    1104
Asp Met Phe Arg Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met
        355                 360                 365

ctt ctg tcc gtt tgc cgg tcg tgg gcg gca tgg tgc aag ttg aat aac    1152
Leu Leu Ser Val Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn
    370                 375                 380

cgg aaa tgg ttt ccc gca gaa cct gaa gat gtt cgc gat tat ctt cta    1200
Arg Lys Trp Phe Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu
385                 390                 395                 400

tat ctt cag gcg cgc ggt ctg gca gta aaa act atc cag caa cat ttg    1248
Tyr Leu Gln Ala Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu
                405                 410                 415

ggc cag cta aac atg ctt cat cgt cgg tcc ggg ctg cca cga cca agt    1296
Gly Gln Leu Asn Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser
            420                 425                 430

gac agc aat gct gtt tca ctg gtt atg cgg cgg atc cga aaa gaa aac    1344
Asp Ser Asn Ala Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn
        435                 440                 445

gtt gat gcc ggt gaa cgt gca aaa cag gct cta gcg ttc gaa cgc act    1392
Val Asp Ala Gly Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr
    450                 455                 460

gat ttc gac cag gtt cgt tca ctc atg gaa aat agc gat cgc tgc cag    1440
Asp Phe Asp Gln Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln
465                 470                 475                 480

gat ata cgt aat ctg gca ttt ctg ggg att gct tat aac acc ctg tta    1488
Asp Ile Arg Asn Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu
                485                 490                 495

cgt ata gcc gaa att gcc agg atc agg gtt aaa gat atc tca cgt act    1536
Arg Ile Ala Glu Ile Ala Arg Ile Arg Val Lys Asp Ile Ser Arg Thr
            500                 505                 510

gac ggt ggg aga atg tta atc cat att ggc aga acg aaa acg ctg gtt    1584
Asp Gly Gly Arg Met Leu Ile His Ile Gly Arg Thr Lys Thr Leu Val
        515                 520                 525

agc acc gca ggt gta gag aag gca ctt agc ctg ggg gta act aaa ctg    1632
Ser Thr Ala Gly Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu
    530                 535                 540

gtc gag cga tgg att tcc gtc tct ggt gta gct gat gat ccg aat aac    1680
Val Glu Arg Trp Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn
545                 550                 555                 560

tac ctg ttt tgc cgg gtc aga aaa aat ggt gtt gcc gcg cca tct gcc    1728
Tyr Leu Phe Cys Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala
                565                 570                 575
```

acc agc cag cta tca act cgc gcc ctg gaa ggg att ttt gaa gca act 1776
Thr Ser Gln Leu Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr
580 585 590

cat cga ttg att tac ggc gct aag gat gac tct ggt cag aga tac ctg 1824
His Arg Leu Ile Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu
595 600 605

gcc tgg tct gga cac agt gcc cgt gtc gga gcc gcg cga gat atg gcc 1872
Ala Trp Ser Gly His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala
610 615 620

cgc gct gga gtt tca ata ccg gag atc atg caa gct ggt ggc tgg acc 1920
Arg Ala Gly Val Ser Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr
625 630 635 640

aat gta aat att gtc atg aac tat atc cgt aac ctg gat agt gaa aca 1968
Asn Val Asn Ile Val Met Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr
645 650 655

ggg gca atg gtg cgc ctg ctg gaa gat ggc gat ggt atc gaa ggt cgt 2016
Gly Ala Met Val Arg Leu Leu Glu Asp Gly Asp Gly Ile Glu Gly Arg
660 665 670

ggt agc gct tgg cgt cac ccg cag ttc ggt ggt taataa 2055
Gly Ser Ala Trp Arg His Pro Gln Phe Gly Gly
675 680

<210> 21
<211> 683
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: DNA sequence
coding for a fusion protein VP22creStrepTag

<400> 21
Met Thr Ser Arg Arg Ser Val Lys Ser Gly Pro Arg Glu Val Pro Arg
1 5 10 15

Asp Glu Tyr Glu Asp Leu Tyr Tyr Thr Pro Ser Ser Gly Met Ala Ser
20 25 30

Pro Asp Ser Pro Pro Asp Thr Ser Arg Arg Gly Ala Leu Gln Thr Arg
35 40 45

Ser Arg Gln Arg Gly Glu Val Arg Phe Val Gln Tyr Asp Glu Ser Asp
50 55 60

Tyr Ala Leu Tyr Gly Gly Ser Ser Ser Glu Asp Asp Glu His Pro Glu
65 70 75 80

Val Pro Arg Thr Arg Arg Pro Val Ser Gly Ala Val Leu Ser Gly Pro
85 90 95

Gly Pro Ala Arg Ala Pro Pro Pro Pro Ala Gly Ser Gly Gly Ala Gly
100 105 110

Arg Thr Pro Thr Thr Ala Pro Arg Ala Pro Arg Thr Gln Arg Val Ala
115 120 125

Ser Lys Ala Pro Ala Ala Pro Ala Ala Glu Thr Thr Arg Gly Arg Lys
130 135 140

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Ser Ala Gln Pro Glu Ser Ala Ala Leu Pro Asp Ala Pro Ala Ser Thr
145                 150                 155                 160

Ala Pro Thr Arg Ser Lys Thr Pro Ala Gln Gly Leu Ala Arg Lys Leu
                165                 170                 175

His Phe Ser Thr Ala Pro Pro Asn Pro Asp Ala Pro Trp Thr Pro Arg
            180                 185                 190

Val Ala Gly Phe Asn Lys Arg Val Phe Cys Ala Ala Val Gly Arg Leu
        195                 200                 205

Ala Ala Met His Ala Arg Met Ala Ala Val Gln Leu Trp Asp Met Ser
    210                 215                 220

Arg Pro Arg Thr Asp Glu Asp Leu Asn Glu Leu Leu Gly Ile Thr Thr
225                 230                 235                 240

Ile Arg Val Thr Val Cys Glu Gly Lys Asn Leu Leu Gln Arg Ala Asn
                245                 250                 255

Glu Leu Val Asn Pro Asp Val Val Gln Asp Val Asp Ala Ala Thr Ala
            260                 265                 270

Thr Arg Gly Arg Ser Ala Ala Ser Arg Pro Thr Glu Arg Pro Arg Ala
        275                 280                 285

Pro Ala Arg Ser Ala Ser Arg Pro Arg Arg Pro Val Glu Gly Thr Glu
    290                 295                 300

Leu Gly Ser Thr Ser Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His
305                 310                 315                 320

Ser Gly Gly Arg Met Ser Asn Leu Leu Thr Val His Gln Asn Leu Pro
                325                 330                 335

Ala Leu Pro Val Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met
            340                 345                 350

Asp Met Phe Arg Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met
        355                 360                 365

Leu Leu Ser Val Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn
    370                 375                 380

Arg Lys Trp Phe Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu
385                 390                 395                 400

Tyr Leu Gln Ala Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu
                405                 410                 415

Gly Gln Leu Asn Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser
            420                 425                 430

Asp Ser Asn Ala Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn
        435                 440                 445

Val Asp Ala Gly Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr
    450                 455                 460

Asp Phe Asp Gln Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln
465                 470                 475                 480
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Asp Ile Arg Asn Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu
                485   490   495

Arg Ile Ala Glu Ile Ala Arg Ile Arg Val Lys Asp Ile Ser Arg Thr
            500   505   510

Asp Gly Gly Arg Met Leu Ile His Ile Gly Arg Thr Lys Thr Leu Val
        515   520   525

Ser Thr Ala Gly Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu
    530   535   540

Val Glu Arg Trp Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn
545   550   555   560

Tyr Leu Phe Cys Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala
                565   570   575

Thr Ser Gln Leu Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr
            580   585   590

His Arg Leu Ile Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu
        595   600   605

Ala Trp Ser Gly His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala
    610   615   620

Arg Ala Gly Val Ser Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr
625   630   635   640

Asn Val Asn Ile Val Met Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr
                645   650   655

Gly Ala Met Val Arg Leu Leu Glu Asp Gly Asp Gly Ile Glu Gly Arg
            660   665   670

Gly Ser Ala Trp Arg His Pro Gln Phe Gly Gly
        675   680

<210> 22
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:synthetic TAT
      protein

<400> 22
Ala Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg
  1   5   10

<210> 23
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic TAT
      protein

<400> 23
Tyr Ala Arg Lys Ala Arg Arg Gln Ala Arg Arg
1 5 10

<210> 24
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic TAT protein

<400> 24
Tyr Ala Arg Ala Ala Ala Arg Gln Ala Arg Ala
1 5 10

<210> 25
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic TAT protein

<400> 25
Tyr Ala Arg Ala Ala Arg Arg Ala Ala Arg Arg
1 5 10

<210> 26
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic TAT protein

<400> 26
Tyr Ala Arg Ala Ala Arg Arg Ala Ala Arg Ala
1 5 10

<210> 27
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic TAT protein

<400> 27
Tyr Ala Arg Arg Arg Arg Arg Arg Arg Arg Arg
1 5 10

<210> 28
<211> 11
<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic TAT protein

<400> 28
Tyr Ala Ala Ala Arg Arg Arg Arg Arg Arg Arg
 1   5   10

<210> 29
<211> 4960
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: vector pCMV-I-Cre-pA

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tagtaatcaa ttacggggtc attagttcat agcccatata tggagttccg cgttacataa 120
cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt gacgtcaata 180
atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca atgggtggac 240
tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc aagtacgccc 300
cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta catgacctta 360
tgggactttc ctacttggca gtacatctac gtattagtca tcgctattac catggtgatg 420
cggttttggc agtacatcaa tgggcgtgga tagcggtttg actcacgggg atttccaagt 480
ctccacccca ttgacgtcaa tgggagtttg ttttggcacc aaaatcaacg ggactttcca 540
aaatgtcgta acaactccgc cccattgacg caaatgggcg gtaggcgtgt acggtgggag 600
gtctatataa gcagagctct ctggctaact agagaaccca ctgcttactg gcttatcgaa 660
attaatacga ctcactatag ggagacccaa gctgactcta gacttaatta agcgttgggg 720
tgagtactcc ctctcaaaag cgggcatgac ttctgcgcta agattgtcag tttccaaaaa 780
cgaggaggat ttgatattca cctggcccgc ggtgatgcct ttgagggtgg ccgcgtccat 840
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ggatagtgaa acaggggcaa tggtgcgcct gctggaagat ggcgattagc cattaacgcg 2040
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tacaatctgc tctgatgccg catagttaag ccagccccga cacccgccaa cacccgctga 2580

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<223> Description of Artificial Sequence: primer

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<220>
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<223> Description of Artificial Sequence: primer

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Claims

1. Use of a fusion protein comprising
(a) a site-specific DNA recombinase domain and
(b) a protein transduction domain (PTD)
for preparing an agent for inducing target gene alterations in a living organism or cell culture, wherein said living organism carries at least one or more recognition sites for said site-specific DNA recombinase integrated in an endogenous gene.

2. The use of claim 1, wherein the PTD is not derived from Antennapedia and preferably is a PTD derived from the VP22 protein of HSV or from the TAT protein of HIV.

3. Use of a fusion protein comprising
(a) a site-specific DNA recombinase domain and
(b) a protein transduction domain (PTD) being not derived from Antennapedia and preferably being derived from the VP22 protein of HSV or from the TAT protein of HIV
for preparing an agent for inducing target gene alterations in a living organism or cell culture, wherein said living organism carries at least one or more recognition sites for said site-specific DNA recombinase integrated in its genome.

4. The use of claim 3, wherein the recognition sites for said site specific recombinase is present within an endogenous gene or a transgene.

5. The use of any one of claims 2 to 4, wherein the TAT protein comprises
(i) the amino acid sequence YGRKKRRQRRR (SEQ ID NO: 10) or a mutant thereof including
(ii) peptides having the amino sequences

AGRKKRRQRRR (SEQ ID NO:22)
YARKARRQARR (SEQ ID NO:23)
YARAAARQARA (SEQ ID NO:24)
YARAARRAARR (SEQ ID NO:25)
YARAARRAARA (SEQ ID NO:26)
YARRRRRRRRR (SEQ ID NO:27)
YAAARRRRRRR (SEQ ID NO:28);
preferably the TAT protein consists of one of the sequences shown in (i) or (ii) above.

6. The use of any one of claims 2 to 4, wherein the VP22 protein comprises the amino acid 16-157 of SEQ ID NO:14.

7. The use of any one of claims 1 to 6, wherein the site-specific DNA recombinase domain is selected from a recombinase protein derived from Cre, Flp, ϕC31 recombinase, and R recombinase and preferably is Cre having amino acids 15 to 357 of SEQ ID NO: 2 or Flpe having amino acids 15 to 437 of SEQ ID NO: 4.

8. The use of any one of claims 1 to 7, wherein the protein transduction domain is fused to the N-terminal of the site-specific DNA recombinase domain.

9. The use of any one of claims 1 to 8, wherein the protein transduction domain is fused to the site-specific DNA recombinase domain through a direct chemical bond or through a linker molecule.

10. The use of any one of claim 9, wherein the linker molecule is a short peptide having 1 to 20, preferably 1 to 10 amino acid residues.

11. The use of any one of claims 1 to 10, wherein said fusion protein further comprises additional functional sequences.

12. The use of claim 1, wherein the fusion protein has the sequence shown in SEQ ID NOs: 2, 4, 6 or 8.

13. The use of any one of claims 1 to 12, wherein the living organism is a vertebrate, preferably a rodent or a fish.

14. A method for inducing gene alterations in a living organism which comprises administering to said living organism, a fusion protein comprising a site-specific DNA recombinase domain and a protein transduction domain as defined in claims 1 to 12, wherein said living organism carries at least one or more recognition sites for said site-specific DNA recombinase integrated in its genome.

15. A fusion protein comprising

(a) a site-specific DNA recombinase domain as defined in claims 2 to 9 and

(b) a protein transduction domain (PTD) as defined in claims 2 to 9

provided that when (a) is the wild-type Flp or Cre then (b) is not the full length VP22 protein of HSV.

16. The fusion of claim 15, wherein the (PTD) is derived from the TAT protein of HIV.

17. A DNA sequence coding for the fusion protein of claim 15 or 16, said DNA sequence preferably comprising the sequence shown in SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 18 and/or 20.

18. A vector comprising the DNA sequence of claim 17.

19. A host cell transformed with the vector of claim 18 and/or comprising the DNA of claim 17.

20. A method for producing the fusion protein of claim 15 which comprises culturing the transformed host cell of claim 19 and isolating the fusion protein.

21. An injectable composition comprising the fusion protein as defined in claims 1 to 12 or 15 to 16.

Fig. 1

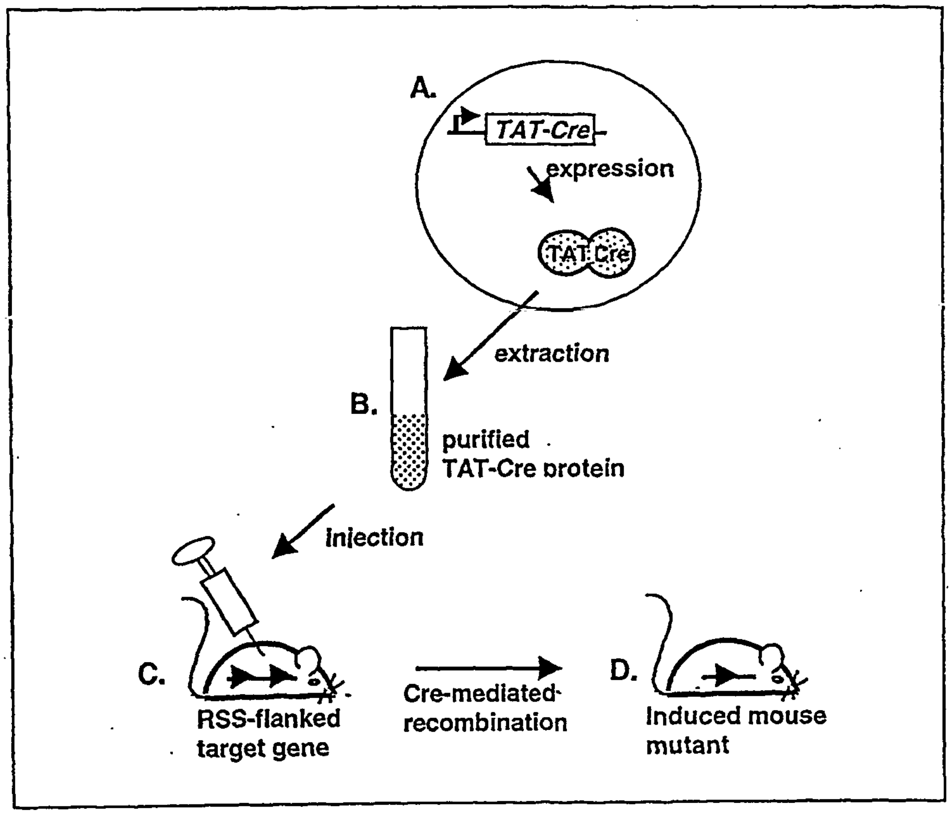
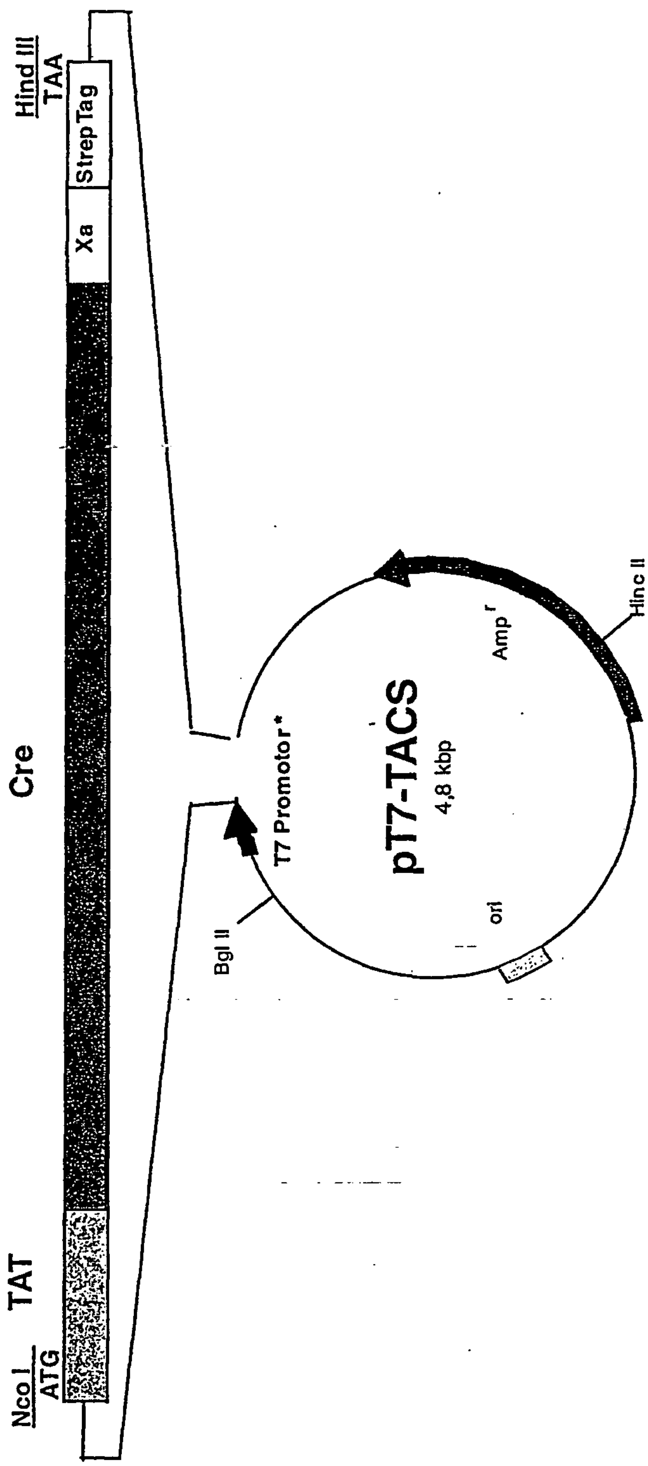
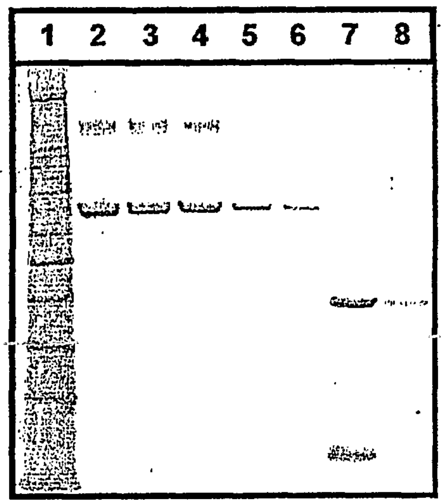

A.
TAT-Cre
expression
TAT Cre
extraction
B.
purified
TAT-Cre protein
injection
C.
RSS-flanked
target gene
Cre-mediated
recombination
D.
Induced mouse
mutant

Fig. 2


Nco I
ATG
TAT
Cre
Xa
StrepTag
Hind III
TAA
T7 Promotor*
Bgl II
pT7-TACS
4,8 kbp
Amp r
Hinc II
ori



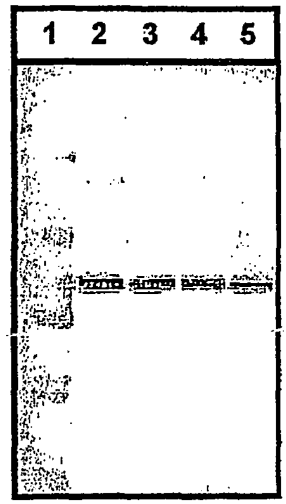


Figure 3

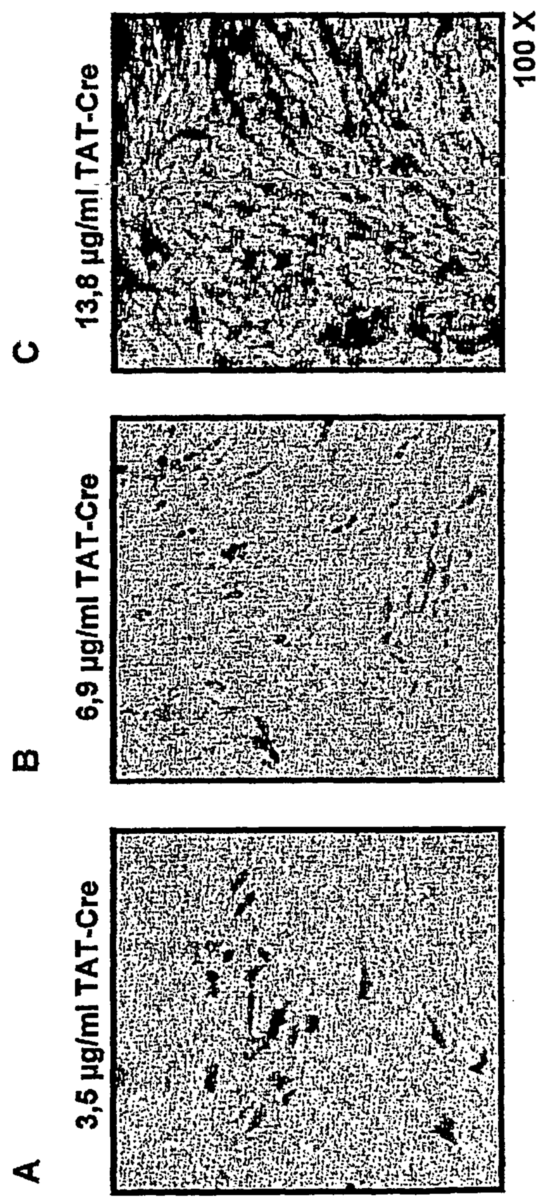
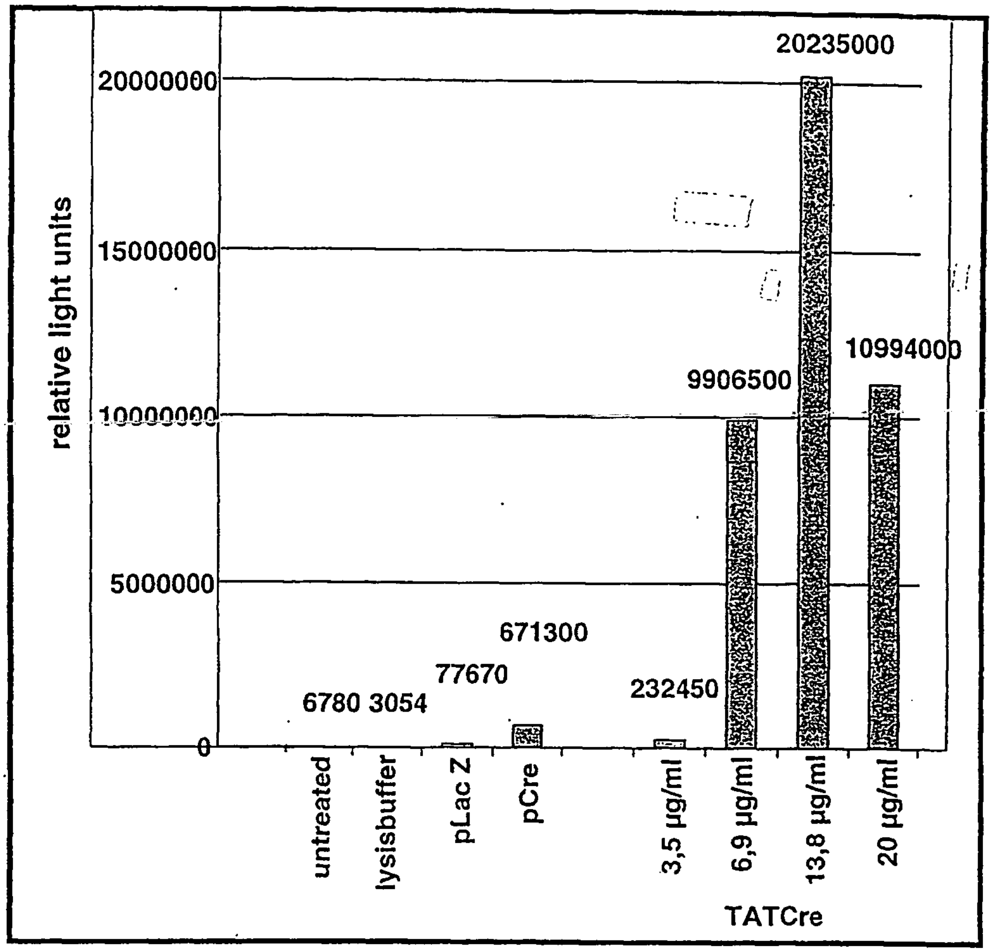


Figure 4

Fig. 5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10000000
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10994000
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6780 3054
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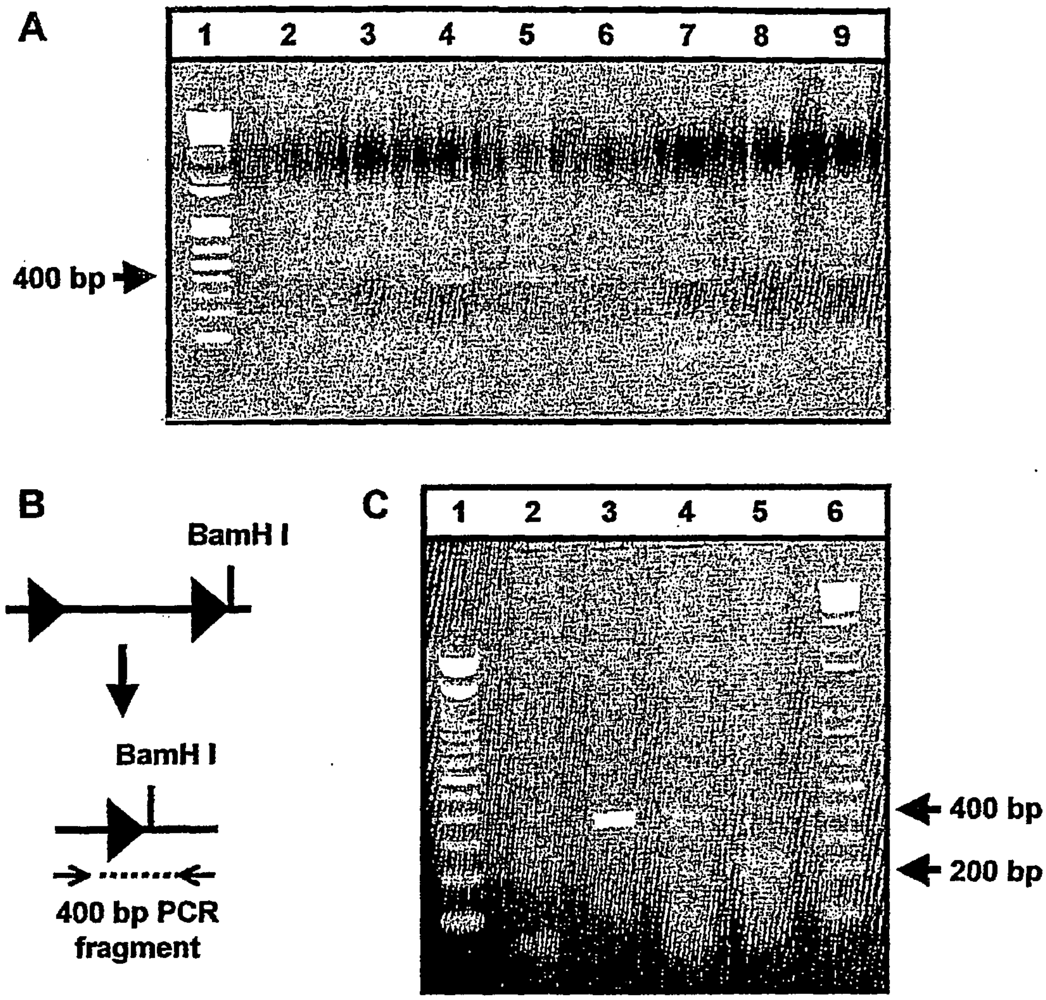
pLac Z
pCre
3,5 µg/ml
6,9 µg/ml
13,8 µg/ml
20 µg/ml
TATCre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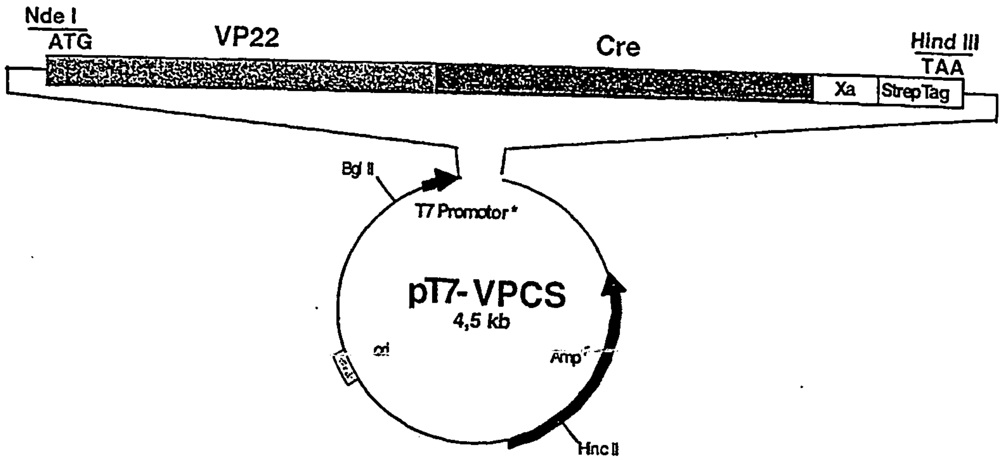
1 2 3 4 5 6 7 8 9
400 bp
B
BamH I
BamH I
400 bp PCR fragment
C
1 2 3 4 5 6
400 bp
200 bp

Figure 6

Fig. 7

A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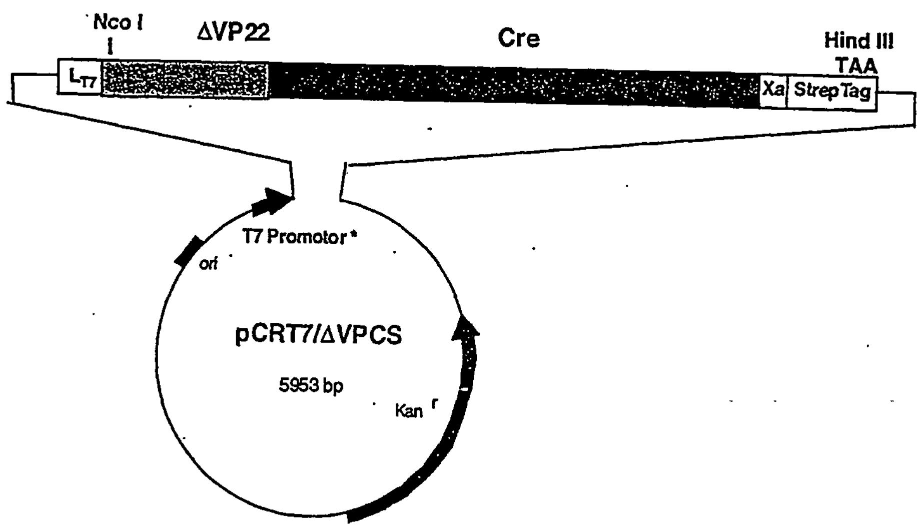
VP22
Cre
Hind III
TAA
Xa
StrepTag
Bgl II
T7 Promotor*
pT7-VPCS
4,5 kb
ori
Hnc II

B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Hind III
TAA
Xa
StrepTag
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ori
pCRT7/ΔVPCS
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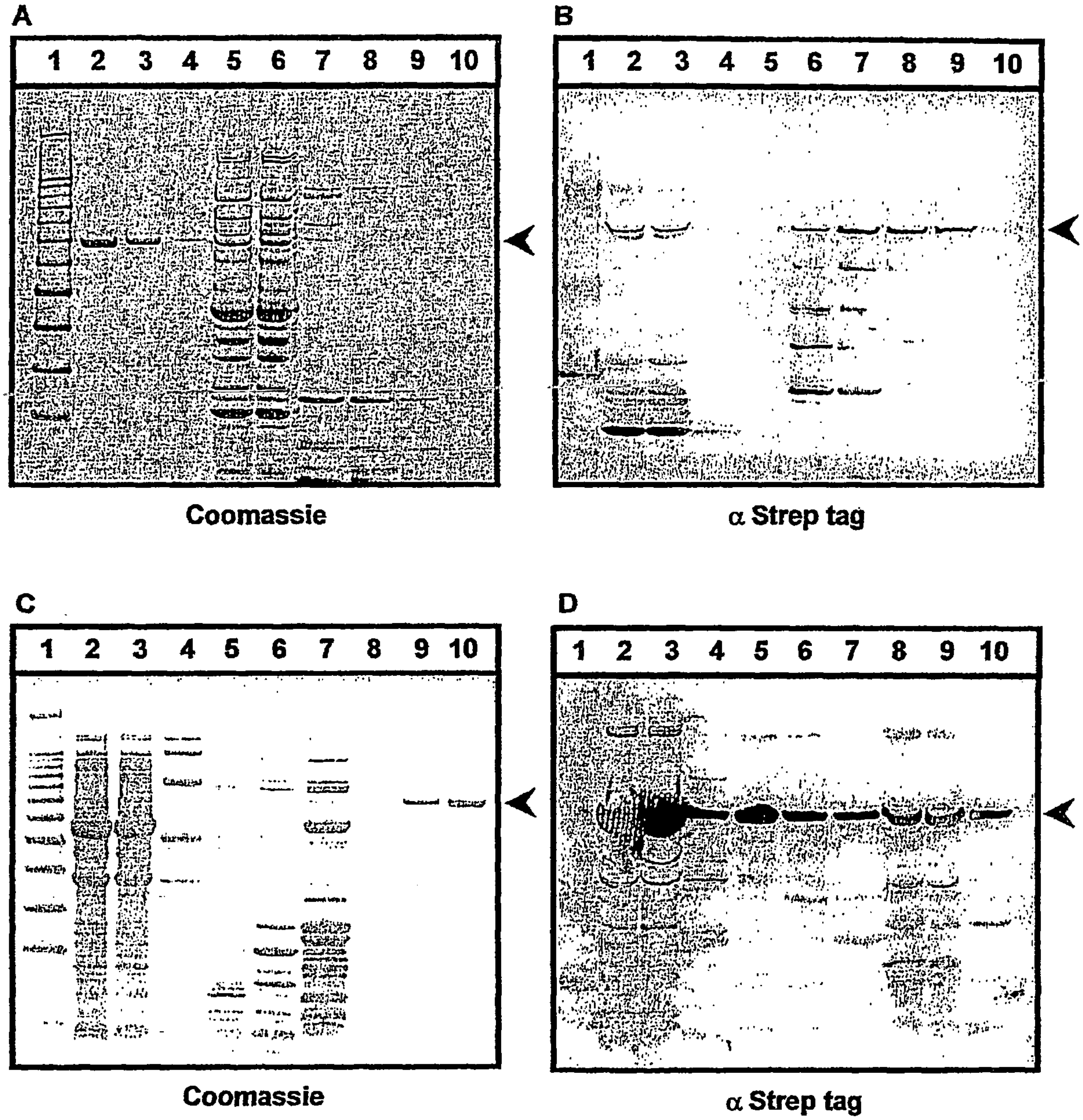
Kan r


A
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Coomassie
B
1 2 3 4 5 6 7 8 9 10
α Strep tag
C
1 2 3 4 5 6 7 8 9 10
Coomassie
D
1 2 3 4 5 6 7 8 9 10
α Strep tag

Figure 8

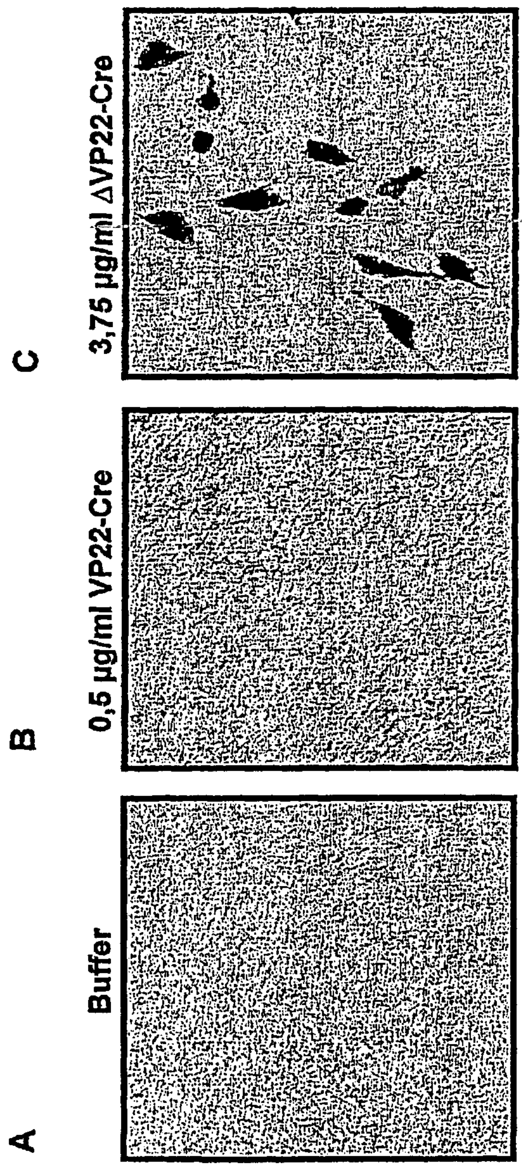
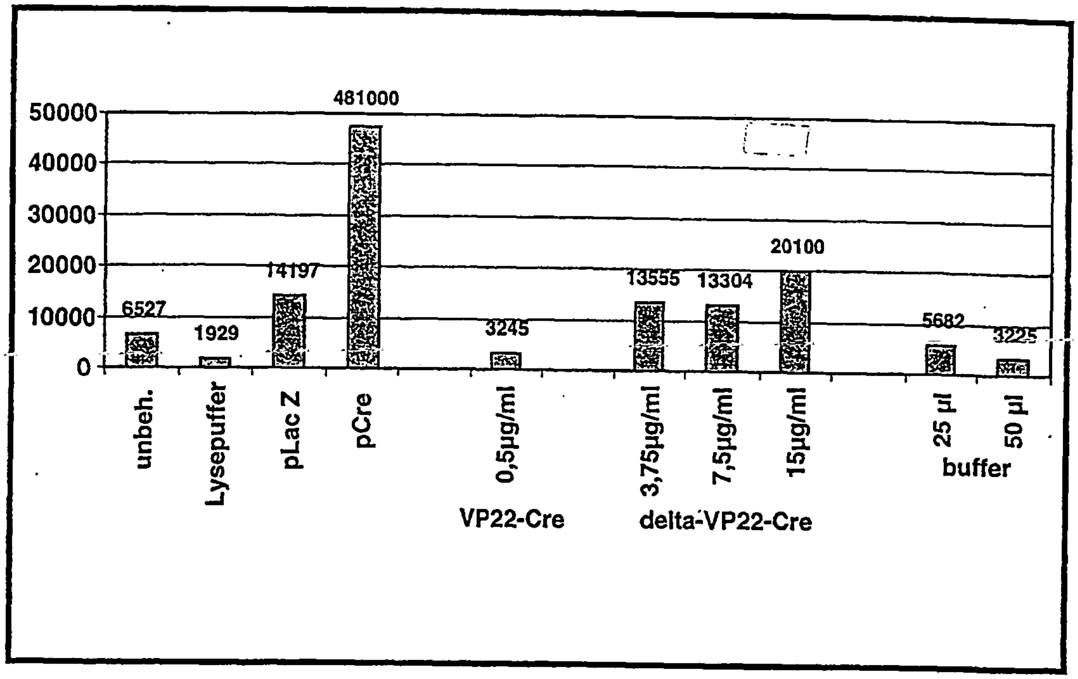


Figure 9

Fig. 10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3225
unbeh.
Lysepuffer
pLac Z
pCre
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3,75µg/ml
7,5µg/ml
15µg/ml
25 µl
50 µl
buffer
VP22-Cre
delta-VP22-Cre

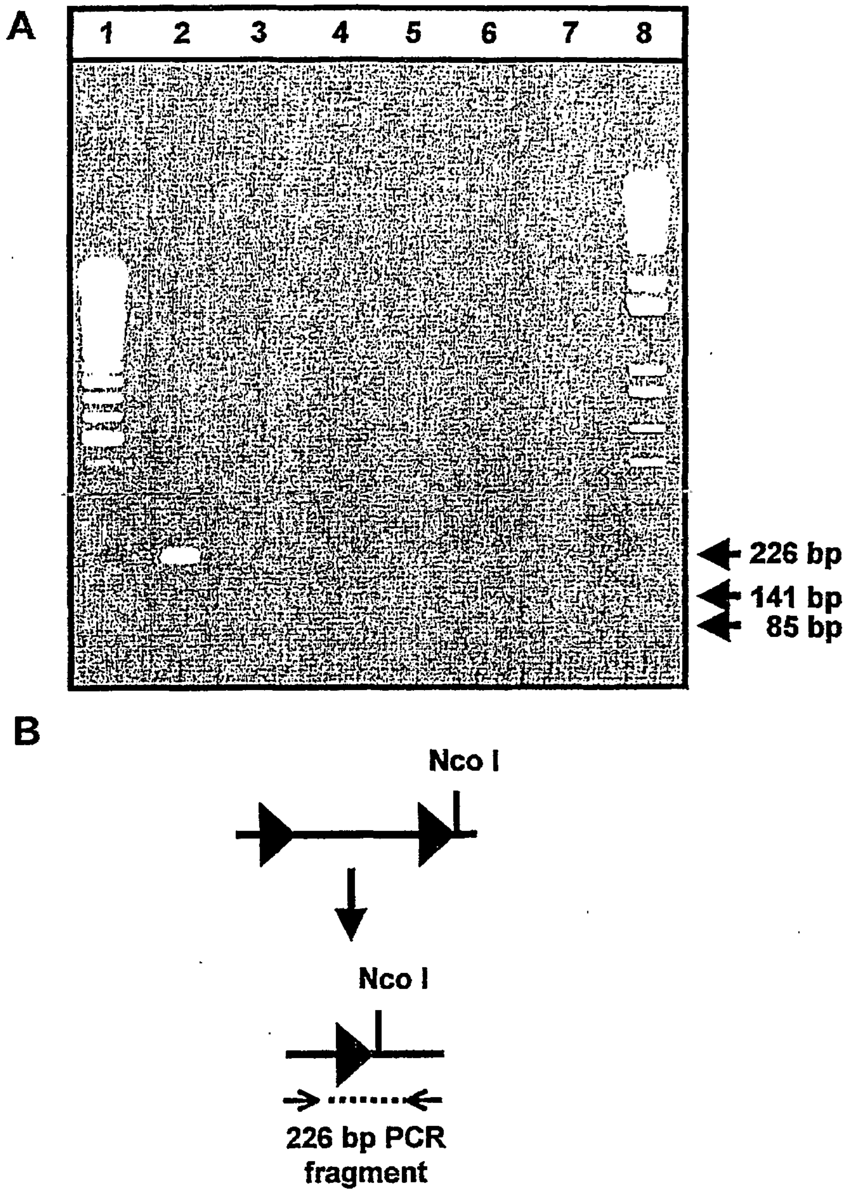

Figure 11